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Journal of DENTAL RESEARCH

Founded by William J. Gies in 1919

OFFICIAL PUBLICATION OF
THE INTERNATIONAL ASSOCIATION
FOR DENTAL RESEARCH

Editor
RANK J. ORLAND

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INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

Organized: New York City, Dec. 10, 1920. Of those at the meeting of organization, the following remain active members: Adolph Berger, Theodor Blum, G. W. Clapp, A. H. Merritt, B. B. Palmer, L. M. Waugh.

Objects (Const., Art. II): The association has been established to promote broadly the advancement of research in all branches of dental science and in the related phases of the sciences that contribute directly to the development of oral health service, and as a whole; and further, to encourage and facilitate cooperative effort and achievement by, and mutual helpfulness among, investigators in all nations in every division of stomatology to the end that dentistry may render cumulatively more perfect service to humanity.

Membership: Total (October, 1958), 938. *Eligibility (Const., Art. III, Sec. 1).*—"Any person who has conducted, and published an account of, a meritorious original investigation in dental science, or in any of the sciences contributory to oral health, shall be eligible to membership in this Association, providing such person conforms to the recognized standards of professional ethics." *Election.*—New members may be elected *only at general meetings of the Association.* Membership in a section is secondary to membership in the Association as a whole. Removal of members from one geographic center to another does not affect their membership in the Association.

Nomination blanks for membership in this Association may be obtained on request from the Secretary-Treasurer.

Endowment fund of the International Association for Dental Research (as of October, 1958): $1,891.01. There have been no expenditures from this fund.

THE JOURNAL OF DENTAL RESEARCH

This JOURNAL is an official publication of the International Association for Dental Research. It is dedicated to the dissemination of knowledge pertaining to the oral cavity, its contents and contiguous parts, and its relation to and effect on the total human organism. The JOURNAL publishes original investigations in these and related fields. Articles are accepted only if they are contributed solely to this JOURNAL.

The JOURNAL is published bimonthly. Subscription price for the volume (6 copies beginning with the January-February issue) is as follows: United States and its possessions, $10.00; (Students, $5.00). Canada, Latin America and Spain, $10.50; (Students, $5.50). Other countries $11.00; (Students, $6.00). Single copies $2.50, postpaid. The subscription fee for members of the IADR is included in the yearly dues paid to the Association. The price of volumes and single numbers of earlier years will be quoted upon request to Dr. David F. Mitchell, University of Indiana, 1121 W. Michigan St., Indianapolis, Indiana.

Matters pertaining to subscriptions, lost copies, change of address, reprints (up to 500), cost of colored reproductions, advertising space, and other business matters should be addressed to The C. V. Mosby Company, Publishers, 3207 Washington Blvd., St. Louis, Missouri.

All manuscripts and correspondence should be addressed to the Editor: Dr. F. J. Orland, Zoller Dental Clinic, University of Chicago, 950 E. 59th Street, Chicago 37, Illinois.

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*These were the Supporting Associates who had made contributions in 1959 at press time to the I.A.D.R. Treasury for support of the JOURNAL. These contributions are in multiples of one hundred dollars.

INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH ANNOUNCEMENT OF 1960 GENERAL MEETING

The 38th General Meeting of the International Association for Dental Research will convene at the Morrison Hotel, Chicago, Illinois, on Thursday, Friday, Saturday, and Sunday, March 17, 18, 19, and 20, 1960. The Dental Materials Group of the Association will meet on Friday, Saturday, and Sunday, March 18, 19, and 20, 1960. The American Association of Dental Schools will meet at the same place beginning Monday, March 21, 1960.

Notice to Prospective Program Participants.—Abstracts of items for the program must be limited to *250 words,* including title, authors' names, etc. *Three copies* are required. The abstract as received must be suitable for insertion, without editing, in the Preprinted Abstracts which will be sent to members before the meeting. This will not constitute a publication, but is only a convenience for the meeting. The abstracts will be published in the JOURNAL OF DENTAL RESEARCH after the meeting as in the past.

There will be no Read-by-Title section of the program. Instead, the editor is accepting short research notes for early publication in the JOURNAL OF DENTAL RESEARCH.

Abstracts entered in the *Edward H. Hatton Award Competition* should conform to the specifications stated on the opposite page under "Editorial Views and News" and must be clearly marked as entries for the competition.

The deadline for receipt of program items will be *November 30, 1959.* An abstract received after that date will probably not be included in the preprinted abstracts and will appear on the program only if a place can conveniently be found for it.

DAN Y. BURRILL, *Secretary-Treasurer*
Northwestern University Dental School
311 E. Chicago Avenue
Chicago 11, Illinois

EDITORIAL VIEWS AND NEWS

The A.D.A. Centennial.—So much has already been written about the Centennial of the American Dental Association that little more can or need be added. It should be emphasized, however, that this 100th Anniversary Meeting in New York City, now only a few weeks away, indubitably will be one of the most significant gatherings ever held anywhere of individuals concerned directly or indirectly with health of the oral area. Since prevention of oral disease is becoming the most important facet of dentistry, a tremendous motivation toward research on the part of the dental profession has become apparent in the latter part of this century of dental progress and certainly is expected to continue on all fronts into the second century of organized dental activity.

Among the many meetings scheduled at the time of the annual session of the A.D.A., there is to be an International Conference on Oral Biology co-sponsored by the International Association for Dental Research. This 3-day conference, beginning September 7 and supported by a Colgate-Palmolive grant, will bring together investigators from many parts of the world to discuss advances made possible by research endeavor in the area of enamel, biochemical and nutritional factors in dental caries, oral soft tissues, oral environment, and bone. The I. A. D. R. also is co-sponsoring the afternoon session of an International Conference on Dental Education and Dental Research, September 11, in which research activities in countries abroad will be described.

Symposium on Caries Evaluation.—Although not directly related to the Centennial meeting in time, place, or sponsorship, there is to be held a Symposium on the Clinical Estimation of Caries Incidence and Increments. This significant gathering at Ohio State University, September 23 and 24, is sponsored by the Study Section of the National Institute of Dental Research of the U. S. Public Health Service. It has for its aim the bringing together of international experts from the fields of epidemiology and clinical investigation in the hope that advances in knowledge made by each group can be shared by all interested in this difficult field of research. A unique feature will be the re-examination of patients initially observed for caries a year ago by seven examining teams from various parts of the United States. This will be followed by a discussion of means of standardizing examination methods and then by a discussion on experimental design. The symposium will have its contents published and thus be available to everyone concerned with this critical area of human caries research.

Historical Aspects of Journals.—In connection with the A.D.A. Centennial, the history of dental publications has been cited as evidence of progress in dental journalism. It is, therefore, appropriate to mention that a history of our JOURNAL OF DENTAL RESEARCH is portrayed by its recent past editor, H. B. G. Robinson, in his Presidential address published on page 640 of this JOURNAL number. Recent issues of the Journal of the American Dental Association have carried exceedingly valuable historical references to dental research development in the United States, and members of the I. A. D. R. should be aware of them.

FRANK J. ORLAND
EDITOR

PRESIDENTIAL INAUGURAL ADDRESS

THE JOURNAL OF DENTAL RESEARCH 1919-1958

HAMILTON B. G. ROBINSON

The University of Kansas City, School of Dentistry, Kansas City, Mo.

THE INCOMING president of this Association is presented with an unusual opportunity—that of addressing a captive audience without limitations on subject or time. With few exceptions, past presidents have taken full advantage of this situation and their subjects have varied from travelogues to erudite scientific discussions. Having served this Association as an officer of one sort or another since 1936, I shall use this opportunity to reminisce and to document the history of our JOURNAL. Having served in the capacity of Editor for more years than any other person, I thought, at first, that I could write a history of the years since 1936 from memory. Soon I found that, unless I checked each year through published reports of the Publication Committee and the Editor and through letters and communiques in my files, I was apt to look at different events through glasses of varying hues. For this reason I feel this discussion will be historically correct, even though less colorful than a straight narration might have been.

To go back before my time as Editor, we can begin at the birth of the JOURNAL. Dr. William J. Gies, then Professor of Biochemistry at Columbia University, held research in high esteem as the "mainspring in the chronometer of science," and he considered dental journalism of the day as the lowest of the low from the viewpoint of professional ethics. He stated that "Dentistry has been asleep in the field of original literature, narcotized by a system of dominant trade journalism that has been notable in the history of dentistry for commercial efficiency, professional obtundity and unlimited superficialty—a system of journalism which, because of its general acceptance and approval by dentists, has demoralized the spirit and impoverished the imagination of dentistry; a system of journalism that has been completely eliminated from respect and influence in every other profession, because of that system's inherent insincerity, unreliability and selfishness."[1]

Gies further stated[1] that "The continued domination of the influences of supply-houses in dental journalism, suggests . . . that dentistry is more a trade than a profession." One is hard put to discover whether the founder of the JOURNAL was more *for* a scientific journal or *against* "trade journalism." Be that as it may, Gies wrote that "THE JOURNAL OF DENTAL RESEARCH is an open break with the past in dental journalism and a move in the direction of complete professional ascendancy in dentistry" and that "THE JOURNAL OF DENTAL RESEARCH

has been conceived in scientific altruism, born of the spirit of dental progress, and nurtured on the ideals of public service; and it is dedicated unreservedly and without qualification to truth and advancement in dentistry, and to the promotion of human welfare through the enrichment and development of that great profession for prevention, alleviation and cure of diseases of the mouth and teeth." Continuing, he announced "THE JOURNAL OF DENTAL RESEARCH will be financed, as a University is supported, with public spirited gifts for this purpose and from a cumulative, permanent endowment fund to be created; also from subscriptions from its successive volumes—not from advertisements of goods for sale."[1]

The JOURNAL was founded then, in 1919, continuing the "spiritual existence of the *Journal of the Allied Dental Societies* which was founded in 1906 as a protest against supply-house dental literature."[1] The first volume of the JOURNAL OF DENTAL RESEARCH lists seventy-four editors and twenty-six subcommittees. William J. Gies was the "Executive Officer of the Board of Editors." The printer was Waverly Press and copyright was held by the JOURNAL OF DENTAL RESEARCH, INCORPORATED, not by any of the supporting societies. By the time the first issue appeared, thirty-three "Founders" had contributed $2,026 to the JOURNAL's endowment fund. It is interesting to note that on May 16, 1919, Dr. Gies wrote[2] to 145 firms and individuals engaged in businesses that dentists are urged to patronize, appealing for financial support for the JOURNAL OF DENTAL RESEARCH.

The JOURNAL continued a relatively uneventful but useful existence. By 1934, there were fifty-six editors listed on its fly page and Dr. Gies still listed himself as "Executive Officer of the Board of Editors." Eleven affiliated societies, each with its representative, included the First District Dental Society of New York, the New York Academy of Dentistry, the American Association of Dental Editors, the Dental Educational Council of America, the Robert R. Andrews Society of Tufts Dental School, and the International Association for Dental Research. The JOURNAL was issued bimonthly and copyright was still held by the JOURNAL OF DENTAL RESEARCH, INCORPORATED. In 1933, Gies wrote[3] that proposals for transfer of ownership were under consideration to (1) the American Dental Association, (2) the International Association for Dental Research with the cooperation of the American College of Dentists, and (3) the First District Dental Society of New York. In the same issue of the JOURNAL, Gies deplored a report, published in the *American Dental Association's Transactions*, to the effect that the editors were contemplating the abandonment of the JOURNAL OF DENTAL RESEARCH and that the First District Dental Society (N. Y.) owned the JOURNAL, but that it was free for the asking and that anyone could take it. Gies branded the statements as untrue and misleading.

In 1933,[4] the eighty-eight editors and representatives of societies voted to transfer ownership as of Jan. 1, 1934, to the International Association for Dental Research *provided* the Association decided to accept the gift.

This was in the depression days of the thirties and the year of the "Bank Holiday." Only a nominal meeting could be held in March but, on May 25, a

"postponed executive meeting" was held in New York City with members from Baltimore, New Haven, New York, Philadelphia, and Pittsburgh, in attendance. Drs. L. M. Waugh, F. S. McKay, and A. H. Merritt were appointed members of a special local committee to consider possible transfer of the JOURNAL's ownership. The Committee recommended acceptance of the JOURNAL, and the Council appointed Waugh, McKay, and Merritt with the President (E. H. Hatton), President-elect (J. L. T. Appleton), Vice-President (T. B. Beust), and Treasurer (B. B. Palmer), a committee of seven, to recommend the number and personnel of the Board of Editors and related matters. The General Secretary (W. J. Gies) obtained pledges of $3,000 and about one hundred subscriptions from eight dental societies to aid the JOURNAL.

At its General Meeting in 1934, this Association voted to accept the JOURNAL, and conditions of further procedure and management were referred to the Council. In June, the Council voted to entrust the affairs of the JOURNAL to a Board of Editors, consisting of one elected representative from each section and the five chief active officers of the Association serving as an acting Board of Editors. Meanwhile, the "existing management . . . for the convenience and protection of the Association" continued to serve with the Acting Board of Editors until the Association should be ready to assume all responsibilities. The Acting Board was composed of J. L. T. Appleton, T. B. Beust, W. G. Skillen, B. B. Palmer, and W. J. Gies.

In 1935, the active editorship was placed in the hands of an Executive Committee of three: T. Rosebury, Editor, E. H. Hatton, Associate Editor, and Paul C. Kitchin, Assistant Editor. W. J. Gies was titled Temporary Second Assistant Editor. At a meeting of the Executive and Finance Committee (A. D. Black, R. W. Bunting, and B. B. Palmer), it was voted that the JOURNAL should carry permanently on its cover the legend "Founded by William J. Gies in 1919," and that certain advertisements be included. Rosebury was "unable effectively to inaugurate his duties as Editor" and resigned as of Dec. 17, 1935.[5] The Finance Committee did not materially increase the resources of the JOURNAL. "The ensuing embarrassments led, after active discussion, to the adoption on March 15, 1936, of the following resolution: Resolved, that the affairs of the JOURNAL OF DENTAL RESEARCH be placed in the hands of a committee to consist of H. C. Hodge, M. K. Hine and H. B. G. Robinson; and that the Board of Editors be continued for the year 1936-37, to make further recommendations at the Fifteenth General Meeting of the International Association for Dental Research."[5]

Because no issues of the JOURNAL had appeared in 1935, it was decided to publish a combined volume for 1935-1936. The new Publication Committee was unusually inexperienced in dental journalism and even less experienced in dental politics. In view of Robinson's experience as editor of the *Penn Dental Journal* two years earlier, while a senior dental student, he assumed primary editorial responsibility. Hall's predental experience in a J. C. Penny's store qualified him to accept much of the business planning. Hine, Hodge, and

Robinson began active work. They had no financial backing (even members were not required to subscribe until 1943), no subscription list, and a backlog of 18 papers, some of them 3 years old.

All the members of the Publication Committee were Rockefeller Fellows at Rochester. Soon, all of the Rochester group in residence rallied like an Irish family at a Donneybrook. In addition to the Publication Committee, Frank Hall, Basil Bibby, Robert Brawley, Marion LeFevre, Dick Manly, Marion McCrea, and Jobe Sedwick pitched in to help and each pulled his oar. Advice was sought on printing, subscriptions, grants, and advertising and even legal advice was obtained. It was soon apparent that the JOURNAL had never officially been given to the Association; in fact, the official transfer of ownership from the JOURNAL OF DENTAL RESEARCH, INCORPORATED, to the International Association for Dental Research never has been consummated. We publish a JOURNAL OF DENTAL RESEARCH which legally may be entirely independent of that published from 1919 until 1934. At any rate, the JOURNAL has been, and is, published, and the copyright held, in the name of the Association. Four issues were published under the name of the Acting Board of Editors and two under the Publication Committee, although, in fact, the Publication Committee assumed responsibility and performed the work of publishing all of them.

It is now amusing that at the 1937 Meeting your speaker reported for the Publication Committee that the chief editorial problem was an accumulation of a backlog of 18 manuscripts. Subscriptions amounting to $1,116 had been received, plus $600 from the American Dental Association, $1,000 from the American College of Dentists, and $1,000 from advertising. As an "expression of confidence," Hall, Hine, Hodge, McCrea, and Robinson were chosen as the second Publication Committee and placed in "full charge of editorial and financial management."[6] This Committee named Robinson as Managing Editor, Hall as Advertising Manager, McCrea as Circulation Manager, Hodge as Treasurer, and Hine as Secretary. During this year, a William J. Gies Endowment Committee for the JOURNAL OF DENTAL RESEARCH was appointed by ad interim action of the Council[7] under chairmanship of Arthur Merritt. Doctors Eby, Kemple, McKay, and Palmer were the other original members. The Committee was instructed to raise a fund to place the JOURNAL on a sound financial basis. The Committee sold "Life Subscriptions" to the JOURNAL for $100 but this embarrassed the Publication Committee since these altruistic life subscribers ceased to contribute to the current operational cost of the JOURNAL, which was on a pay-as-you-go basis. Soon the Endowment Committee recognized this problem and paid for life subscribers at current rates. Later, through misinformation or misunderstanding, or both, the Endowment Fund was changed to the William J. Gies Endowment Fund for the Advancement of Dentistry. After some twenty years, it made its first grant last year to the JOURNAL which it was established to support.

But let us get back to the Rochester Group and the Publication Committee, Hine had gone to Illinois, Robinson to Washington University, McCrea to

Baltimore, and Hall to Medical College of Virginia. That "old gang of mine" was breaking up but not losing its deep interest in the JOURNAL.

By 1938, the Publication Committee consisted of Robinson (Editor), Hall (Business Manager), and McCrea (Circulation Manager). The JOURNAL continued on a pay-as-you-go basis. The Waverly Press and its executives were most kind, as the earlier management had a sizeable unpaid bill when the Rochester Group took over. Waverly Press continued to print the JOURNAL, patiently waiting until the founder and some of his close friends arranged payment of the old debt, a procedure in which the Publication Committee was not permitted to participate. The Editor would write to the Business Manager to ascertain the number of pages that might be included in each successive issue. The cost of publication, including printing, binding, wrapping and mailing, was increasing as America was coming out of its great Depression. The Publication Committee had long sought an angel, for advice of others experienced in publication of the periodicals of scientific societies indicated that few journals of this type survived long on a self-supporting basis. As early as 1937, a proposal to become affiliated with the American Dental Association was made by the Publication Committee through Dr. Harvey Burkhart, then Director of Eastman's Rochester Dental Clinic and an American Dental Association trustee. This plan included appointing a representative of the American Dental Association to the Board of Editors, leaving control of the JOURNAL in the hands of this Association, but considering the JOURNAL OF DENTAL RESEARCH an official journal of the American Dental Association to publish long technical papers submitted to the Journal of the American Dental Association. Subscriptions were to be supplied to American Dental Association members at the current rate ($4.00) for International Association for Dental Research members, and the American Dental Association was to make a grant to the JOURNAL. All of the proposals met with the approval of the American Dental Association's Journal Committee, except that which would have made this JOURNAL an official American Dental Association organ. A grant of $600 was given for one year by the Research Committee of the American Dental Association, but the plan died on the vine.

That same year, 1937, another abortive plan, conceived by Doctors A. D. Black and W. H. G. Logan, was proposed to raise $1,800 per year for the JOURNAL by direct contributions from the schools. This plan did not go beyond making requests to schools. Only one school gave a contribution, as most schools were sorely in need of money themselves. In 1939, through W. J. Gies, a proposal was made by Dental Survey's publishers that they publish and underwrite the cost of publication of the JOURNAL. In 1940, during an investigation of the merits of the proposal by a Committee headed by the Editor, the proposal was reported as being withdrawn.

In 1940, the Constitution of the Association was amended (Article IX, Section 2 A) so that the JOURNAL was to "be conducted for the Association by a Board of Editors," elected by sections, and the "Publication" was placed "in charge of an executive committee of three members." This was the form of

management until 1957, when a new constitution abolished the Board of Editors and placed responsibility for the JOURNAL in the hands of a five-member Publication Committee. In March, 1941, McCrea resigned as Circulation Manager and he was replaced in 1942 by S. S. Arnim, then at the Medical College of Virginia.

In the 1941 volume, two papers that related to dentifrice abrasion were published. One, by Paul C. Kitchin, a past-president of the Association, reported the prevalence of root exposure at various ages and the other, by Richard Manly, presented evidence that abrasive-containing dentifrices could wear away such exposed root surfaces. The Procter and Gamble Company was then selling a nonabrasive, liquid dentifrice. That company requested 50,000 reprints of these two papers, but in view of a policy of the American Association of Dental Editors against supplying any large numbers of reprints, the request was denied by the Publication Committee. A second request was made that the December, 1941, issue of the JOURNAL be distributed to the members of the American Dental Association at the expense of Procter and Gamble. This issue, containing the two articles mentioned, was to be sent out with the advertising, index, and volume's supplementary material removed, but with a subscription blank for the JOURNAL inserted. The Publication Committee, the Association's President, and its General Secretary agreed that the plan was worthy and precautions were taken, such as having the list of American Dental Association members supplied by its Central Office to the printer. No supplemental material was sent with the JOURNAL, although Procter and Gamble sent letters to a similar or identical group of 50,000 dentists directing attention to the two articles. The company had agreed to pay thirty-four cents per issue, somewhat more than it cost to have the issue reproduced in those days of lower printing and paper costs. The Publication Committee was quite proud of itself. It had denied a request to sell large numbers of reprints but had succeeded in getting the JOURNAL, with a subcription blank, in the hands of all American Dental Association members, and had made plans to increase its meager finances. At least once, almost every American dentist would see a journal devoted to dental research and some might become interested. Was this not a cause for pride?

How wrong the Committee was in underestimating the visciousness of misinformation and misinterpretations. At least three editorials and five resolutions criticized the Committee and the JOURNAL, although no effort was made by the critics to get any facts from the members of the Publication Committee. The editor was accused of "prostituting" the JOURNAL[8] and, as one outcome, the Business Manager was directed to return to the company all money except that for the actual cost of printing and mailing the 50,000 copies. The Publication Committee was urged to apologize for its actions and to promise future good behavior. Since the members of the Publication Committee had acted in good faith with the approval of the President and Secretary of the Association, and since it felt that the benefits outweighted any abuses, and since the Editor held that to yield to these groups not directly responsible

for or supporting the JOURNAL would set a precedent for any other pressure group to dictate to or control the JOURNAL which belonged to this Association, there never was any apology.

In 1945, the Editor and other members of the Publication Committee began to make informal inquiries of The C. V. Mosby Company through Mr. John Mosby, its President, Dr. Claude Wood, its Vice-President and a member of this Association, and Mr. G. P. Oberst, its educational representative. The questions of printing, such as performed by the Waverly Press, and of publication were discussed. In effect, the Publication Committee had been publishing the JOURNAL as agents of this Association, but the pressures of increased cost in a spiraling economy left as a serious question just how long this plan could continue. Such agreements are not made overnight but, in 1946, a first step was taken when the Christian Board of Publication in St. Louis became the printer of the JOURNAL.

The Publication Committee, representing the Association, and Mr. Mosby and Dr. Wood, representing the Mosby Company, gradually developed a workable contract. It was submitted to all members of the Board of Editors for suggestions, revisions, or approval. Many revisions were written and in the final stages Dr. Claude Wood, the Editor, and Dr. Gies met in New York City in the Editor's room during a Greater New York Meeting for a final conference. Gies' suggestions that the Mosby Company be known as the "Publisher" and that the Association remain the owner and copyright holder were accepted and the layout for the cover was revised to read "Published for the International Association for Dental Research by The C. V. Mosby Company." Dr. Gies had suggested that the proposed contract be changed so that rather than the Mosby Company assuming all financial responsibility for publishing, printing, mailing, and distribution of the JOURNAL that the Association and the Company share in any profits or losses. The fallacy of this proposal lay in the two facts; namely, that the experience of many years had taught that publishing the JOURNAL was unlikely to be profitable, and neither the Association nor the Publication Committee were in position to assume responsibility for losses. The wisdom of the decision not to put in a "sharing" clause is born out by the reported loss of $24,872 in 10 years' experience of The C. V. Mosby Company with the JOURNAL.[9] The C. V. Mosby Company had proposed paying salaries to the Editor and Business Manager but Gies suggested that a royalty of $150 per month be paid to the International Association for Dental Research, instead. This was done. The possibility of another publishing house developing a similar contract was investigated, but the single other proposal received was considered not to be as good as Mosby's offer. In 1948, The C. V. Mosby Company began to publish the JOURNAL for the Association, the contract appearing in the April, 1948, issue.[10]

Again, as in 1941, a group of dental organizations passed resolutions criticizing the Association and the Publication Committee for its action, this time claiming that the JOURNAL had been "sold" to the Mosby Company. Apparently, the authors of some of these statements did not even take the time

to read the published contract. Since it was also claimed, by groups not directly responsible for the JOURNAL, that the Board of Editors had exceeded its authority, the officers of the Association issued a statement on April 24, 1948, which, in essence, stated:

"The officers find no support for claims that have been made to the effect that the JOURNAL OF DENTAL RESEARCH has been sold to The C. V. Mosby Company for the purpose of commercial exploitation. They hold to the belief that the constitution confers sole and undivided authority on the Board of Editors to conduct the JOURNAL OF DENTAL RESEARCH for the International Association for Dental Research. They find no constitutional support for the hypothesis that decisions of the Board of Editors must be ratified by the Council or the Association before they become operative. They believe that the contract with The C. V. Mosby Company is legal and binding and can be changed only by mutual agreement of that Company and the Board of Editors. They are of the opinion that the Publication Committee and the Board of Editors have made annual reports in complete compliance with the constitutional directive, and that, for the last three occasions at least, these reports have indicated dissatisfaction with the then current publication plan. They are certain that these reports have been formally approved and usually with favorable commendation of the Publication Committee. They anticipate that appropriate reports will be forthcoming at the coming meeting of the Association in June, and that, at this time, it will be in order to open up the matter for further discussion and such action as may be taken within the limits permitted by parliamentary rules and the constitution."[11]

One of the primary points of contention appeared to be the payment of $1,800 annually, as a royalty, to the Association. At the 1948 General Meeting, the Publication Committee was instructed to investigate the possibility of finding a suitable substitute for the royalty, which was being used largely for essential expenses of the editorial and business offices. The changing economic situation was to settle this problem soon.

A communication from the New York Academy of Dentistry, presented at this Association's 1949 General Meeting, proposed that the Association accept a gift of $2,000 per annum for ten years as a replacement for the royalty from the publisher, and that the JOURNAL carry the statement "Published by the International Association for Dental Research, with the cooperation of The C. V. Mosby Company." A committee of the Council drafted a reply[12] rejecting the offer because it presented no advantages over the existing arrangements, and because it was felt that the "form and mode of publication meets all the requirements of a first class, ethical publication and that it is serving admirably its purposes in the promotion of dental research." This statement met with unanimous approval of Council.

With the 1949 volume, the Publication Committee began to purchase additional pages for the JOURNAL with a share of the money received as a royalty. In 1949, 97 extra pages were purchased. In 1950, a new contract with The

C. V. Mosby Company increased the size of the JOURNAL by 300 pages per volume with an increase in subscription rates. During 1951, Arnim resigned as Circulation Manager and was replaced by Paul E. Boyle.

In 1954, The C. V. Mosby Company explained that, in view of appreciable annual deficits from publication of the JOURNAL, it could not continue to pay the annual royalty to the Association. After other organizations, including the University of Chicago Press and dental book publishers, were consulted as possible publishers of the JOURNAL, it was agreed that this portion of the original contract be amended in line with the proposal. As a substitute, the Publication Committee was authorized to seek additional funds from selected groups, individuals, corporations or foundations who might aid the JOURNAL financially as "Supporting Associates." The 1955 volume lists twenty "Supporting Associates" who contributed "in multiples of one hundred dollars" each. The funds from "Supporting Associates" were used, primarily, to pay for extra pages in the JOURNAL.

In 1955, the publishers again reported that heavy deficits left them with a question of whether or not it was feasible for them to continue assuming the annual loss of publication. In the late months of that year Dr. John Hein suggested that the Colgate-Palmolive Company might be interested in sending the JOURNAL to dental students. As a result of this, the Colgate-Palmolive Company began to send the JOURNAL to over 3,000 third-year dental students in all United States Dental Schools. The company paid the student rate to the publisher but sent no advertising to the students. The Editor sent a letter to each student explaining the value of a research journal in dentistry and naming the organization giving the subscription. Again voices were raised in protest and complaint. Again the JOURNAL was able to survive in an expanding economy. The custom of the Colgate-Palmolive Company paying for student subscriptions for third-year dental students has been expanded to include Canada.

In 1957, it was proposed that the Federal Government might aid the publication of the results of dental research, as it was aiding the prosecution of research. As a result, a special committee of the United States Public Health Service Surgeon General's Advisory Council on Dental Research has been investigating the need for support of publication and a grant of $5,000 for one year ($15,000 for five years) has been received through the Advisory Council on Dental Research and the National Institute for Dental Research to support publication of the JOURNAL. Other groups are investigating the possible need for dental research journals.

Let us look at the record. In the years 1919 through 1958, the JOURNAL OF DENTAL RESEARCH has published 2,149 research papers in the field of dentistry. The JOURNAL, which has always existed on a hand-to-mouth basis, has an enviable record which is equalled by few other professional or scientific periodicals. The JOURNAL still exists because of people who have worked for it, and institutions that have supported it. Dentistry as a profession and a

science will be served by continuing and expanding support. Let us work for this before we consider diluting our resources for multiple journals of dental science at various levels of erudition.

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INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

ABSTRACTS OF PAPERS PRESENTED
SHERATON-PALACE, SAN FRANCISCO, CALIF.

MARCH 19-22, 1959

COMPILED BY FRANK J. ORLAND, EDITOR*

I. ODONTOGENESIS. MARCH 19, AFTERNOON, SECTION A.

1. CALCIFICATION OF THE PRIMARY DENTITION: A RE-EVALUATION.—*Bertram S. Kraus, Department of Orthodontics, School of Dentistry, University of Washington, Seattle.* Certain currently held concepts about the phenomena of calcification of the primary teeth have survived unchallenged through many editions of textbooks on oral anatomy, histology, and embryology. The evidence for some of them is either insufficient, inadequate, or even nonexistent. This presentation examines the following three claims: (1) that calcification of the primary teeth commences at 4 or 4½ months in utero, (2) that initial calcification proceeds in regular sequence from central incisor to second molar, and (3) that enamel formation in the incisors and cuspids begins from three calcification centers. A series of 95 human fetuses were cleared and stained with alizarin red S. The teeth were removed and measured, with an error of less than 0.05 mm. All calcified material was stained. The ages of the fetuses ranged from 8 to 18 weeks. It was found that: (1) initial calcification occurred, on the average, at 14 weeks, or 3½ lunar months, (2) initial calcification began in the central incisor, followed in fixed order by the first molar, the lateral incisor, the cuspid, and the second molar, respectively, and (3) there was not more than one center of calcification for the central incisor, the lateral incisor, or the cuspid. These results indicate that careful reconsideration of some of the concepts regarding the ontogeny and the phylogeny of the human dentition is needed.

2. AMELOGENESIS IN THE OPOSSUM.—*Harry E. Frisbie, University of California School of Dentistry, San Francisco.* Partially developed teeth dissected from the crypts of young opossums and prepared without decalcification or small decalcified blocks of tissue were used. Supplemental material was taken from the rat, dog, cat, and pig. Ameloblasts at all stages, preceding and to completion of enamel, showed prominent longitudinal and transverse striae. These fine bands continued laterally between the cells and to the dentin surface through a Tomes's process of full cellular width. At their basal ends they were continuous through intercellular bridges with the cells of the outer reduced parts of the enamel organ. Interspersed along the fibers were numerous basophilic granules. Within the matrix of formal enamel, similarly appearing and oriented fibrous bundles were present. At the outer enamel border, they were continuous with the corresponding cellular elements and within the enamel could be clearly determined to be essentially disposed

*The members of the Program Committee, Hamilton B. G. Robinson, Neb B. Williams, and Dan Y. Burrill, as well as Duane F. Taylor, Program Chairman of the Dental Materials Group, gave invaluable assistance in preparation of these abstracts.

longitudinally to the rods, transversely directed or cut in cross section. Longitudinal bundles were absent from inter-rod areas. The basophilic granules as seen in the cells were missing from Tomes's process and enamel. Stages prior to enamel elaboration showed direct continuity or contiguity between the longitudinal cellular or rod fiber bundles and the peripheral ends of the fibers of the dentin matrix. Where this could be determined no definable basement membrane was observed.

3. MICROGRAPHY AND MICRORADIOGRAPHY OF DENTIN.—*Grant Van Huysen, Indiana University, School of Dentistry, Indianapolis.* Thirty micron thick ground sections of dentin from fully formed but unerupted premolar tooth crowns were photomicrographed with light. The same sections were then x-rayed in contact with fine-grained Eastman Spectrographic films, type 649-0. The x-ray exposures were made with a low voltage Norelco x-ray generator at 3 kv. and 3 Ma. The contact x-ray images of dentin were photomicrographed at 500 × magnification and compared with the light photomicrographs of the same ground sections of dentin. The light micrographs showed dentin tubules surrounded by either a bright or dark ring depending upon the level of the focused 4 mm. objective. The bright and dark ring phenomenon was believed to be due to an optical illusion known as the "Becke Effect." The bright peritubular rings seen in the historadiographs of the ground sections of dentin were probably x-ray diffraction effects, brought about by the crystalline structure of the tissue. The radiolucent peritubular rings did not appear in the historadiographs of the decalcified dentin of these young teeth.

4. THE EFFECT OF HYPOXIA UPON DEVELOPING ENAMEL.—*William F. Via, Jr., William K. Elwood, and Jose Bebin, Henry Ford Hospital, Detroit.* Previous studies have shown a relationship between the occurrence of brain damage and the occurrence of enamel hypoplasia in human beings. An experiment was designed to determine if hypoxia of the mother would cause a disturbance in amelogenesis in the fetus. Pregnant rats were subjected to a mixture of 95 per cent N_2 and 5 per cent CO_2 in a bell jar until unconscious and their respiration became apneustic. The animals were then removed from the chamber and revived. Two pregnant rats were in the tenth day of gestation, 3 were in the fifteenth day of gestation, and 3 were in the twentieth day of gestation. The animals were sacrificed on the twenty-first and twenty-second days of pregnancy. Celloidin serial sections were made of the heads of the young. No difference was found to exist between the litters of the control animals and the litters of those mothers which were subjected to hypoxia on the tenth or fifteenth day of gestation. Litters of the 3 pregnant animals which were subjected to hypoxia on the twentieth day of pregnancy did show defects. Disruption of amelogenesis was evident in one litter and lens defects were seen in the other two litters. Definite brain damage had occurred in only one specimen. The results indicate that the fetus is most susceptible to acute maternal hypoxia late in the gestational period.

5. ALLOXAN DIABETES AND THE ENAMEL PROTEIN MATRIX IN MICE.—*Harold S. Fleming, Howard University, Washington, D. C.* Alloxan diabetes induced alterations in the enamel protein matrix of mandibular incisors in dba Bar Harbor and Rockland mice. Alloxan dosage was 0.2 mg. per gram of body weight from a solution of 400 mg. in 20 c.c. of distilled H_2O. A significant number of male mice but not female mice showed between 0.5 to 1 per cent and occasionally more sugar in their urine after being on CaF_2 at 10 ppm for 40 days. When mice were given alloxan and CaF_2 and there was between 1

and 2 per cent (3 to 4+) sugar in the urine, the zone of matrix secretion in the mandibular incisors was much shorter and the zone of compression, while being altered in appearance, was likewise smaller than those seen with CaF_2 alone or ' Parotin'' (both previously reported). Vascularity adjacent to stratum intermedium cells and in other nearby regions was decreased. With alloxan, where no CaF_2 had been given, the zone of the matrix secretion was extremely small and often the zone of compression became practically nonexistent. With transplants of tooth germs in alloxan-treated mice, these zones were markedly shortened. Furthermore, the zone of the enamel matrix secretion appeared to be inactive. A majority of such transplants were completely absorbed. Male mice were more susceptible to alloxan diabetes, being usually 1 to 2 per cent (3 to 4+). This same degree was difficult to obtain in the females at the doses of alloxan used.

6. Dentin Formation During Exposure to and Withdrawal From Atmospheric Contaminants.—*W. R. Stanmeyer, C. T. G. King, U.S. Naval Medical Research Laboratory, New London, R. A. Colby, U.S. Naval Dental Clinic, Yokosuka, Japan, and H. H. Scofield, U.S. Naval Dental School, Bethesda.* In an atmosphere independent of the earth's air, the effect of contaminants is an important environmental problem. Adaptation and tolerance to certain levels of contaminants has been demonstrated. This work was directed toward the effect of abrupt return to a clean atmosphere on the formation of dentin and blood serum Ca-P ratios. Sexually mature male rats, under carefully controlled experimental conditions, were exposed for periods of up to 15 days to 15 per cent CO_2 in air enriched to 21 per cent O_2. Atmospheric contamination was abruptly ended and the animals were then allowed to recover for periods of up to 15 days. Histologic examination of the dentin of the incisor teeth showed that, during the contaminant phase of the experiment, accentuated pairs of hyper- and hypocalcified incremental lines first appeared at a distance from the pulp corresponding to the date of introduction of CO_2. The recovery phase was accompanied by a band of hypocalcified dentin 1 to 2 days in width. In silver preparations, this band stained darker than the rest of the dentin, suggesting the formation but lack of calcification of the dentin matrix. The dentin pulpal to this band stained more intensely with hematoxylin, suggesting a hypercalcification. In silver preparations, this zone appeared light, which suggested that while the dentin may have been hypercalcified, there were fewer fibrils and a greater amount of cementing substance. Blood serum calcium values showed intermittent hypercalcemias during the exposure to the contaminant with a return to base line values upon recovery. Serum inorganic phosphorus values were lowered during exposure to CO_2 with return to normal values during recovery.

7. Relationship of Hypoplastic Teeth and Surgical Trauma in Cleft Repair.—*John R. Mink, Indiana University, School of Dentistry, Indianapolis.* Observations of the maxillary anterior teeth are now being studied on repaired cleft lip and cleft palate patients to determine the relationship of hypoplastic teeth and surgical trauma occurring during the repair of these congenital defects. The group is composed of 71 subjects with permanent dentition and 20 with primary dentition in the maxillary segment. The degree of hypoplasia is determined by clinical and x-ray examination and then classified as none, mild, moderate, or severe hypoplasia. The type of hypolasia is then related to the original cleft lip and/or palate classification. The results are as follows: Cleft palate patients only—from a sample of 5 in all

classifications, 4 with permanent dentition had no hypoplasia and 1 permanent had moderate hypoplasia. Cleft lip patients only—from a sample of 8 in all classifications, 2 permanent and 1 primary had no hypoplasia, 4 permanent had mild hypoplasia, and 1 permanent had moderate hypoplasia. Cleft lip and palate patients—from a sample of 78 in all classifications in Class II, 2 had no hypoplasia and 2 had mild hypoplasia; in Class III, 6 primary and 2 permanent had no hypoplasia, 5 primary and 7 permanent had mild hypoplasia, 3 primary and 14 permanent had moderate hypoplasia, 5 primary and 12 permanent had severe hypoplasia; in Class IV, 1 permanent had mild hypoplasia, 3 permanent had moderate hypoplasia, and 16 permanent had severe hypoplasia.

8. VASCULAR SUPPLY TO THE DEVELOPING MOLAR TEETH OF RATS.—*Sol Bernick, Department of Anatomy, University of Southern California, Los Angeles.* Young rats, 1 to 26 days old, were used to study the vascular supply of the developing molar teeth and periodontium of rats. The visualization of the vascular patterns of the developing molars was accomplished by various injection methods. The teeth and jaws were fixed in a formol acetic acid alcohol solution. The specimens were decalcified, dehydrated, and embedded in nitrocellulose in the usual manner. The embedded blocks of tissue were sectioned at 100 to 250 μ thicknesses, and the cut sections were cleared by the spalteholz technic. At the beginning of amelogenesis, blood vessels from the oral mucosa invaded the enamel organ and passed toward the ameloblastic layer. A rich capillary network was observed lying directly adjacent to the ameloblasts. This vascular pattern, intimately related to the ameloblasts, persisted in the developing molars until the formative stage immediately preceding eruption was reached. In the earlier stages of tooth development (1 to 10 days), small caliber vessels were most prominent in the pulp. As the tooth continued its development and eruption, there was an increase in the number of large-sized vessels supplying the pulp. The pulpal vessels branched freely in the pulp proper and finally reached the pulpal odontoblastic border, where capillaries arose and penetrated the odontoblastic layer to form a rich vascular plexus directly adjacent to the predentin. In addition, the vascular patterns to the developing periodontal membrane and gingivae were traced.

9. EFFECT OF SODIUM FLUORIDE ON THE DEVELOPING TEETH OF RATS.—*B. R. Bhussry, Georgetown University, School of Medicine and Dentistry, Washington, D. C.* The purpose of this report was to demonstrate the effect of various concentrations of sodium fluoride on the developing teeth of rats. A total of 125 young rats, 3 to 10 days of age, were used in this study. A 0.1 per cent sodium fluoride solution was administered either intraperitoneally or intraorally in doses from 0.10 ml. (50 μg) to 0.25 ml. (125 μg) for a varying number of days. The animals were sacrificed after 2, 3, 5, 7, 10, 12, and 15 days of treatment. Serial sections from 83 animals were cut and stained for histologic examination. Almost all animals receiving sodium fluoride showed abnormalities of amelogenesis. Vacuolization and capillary invasion of the odontoblastic layer with hyperemia of the pulp was a common feature. Calcification and maturation of the enamel and dentin matrix were retarded, and calcification disturbances were evident. The incisor teeth showed more striking changes than the molars in the same mouth. The severity of the effect of sodium fluoride on the developing teeth seemed to depend upon the concentration of the fluoride solution and the state of amelogenesis and dentinogenesis.

9a. Intraoral Transplantation of Teeth.—*R. Gordon Agnew, Chester C. Fong, William S. Giles, and Theodore S. Grant, University of California, Berkeley.* A progress report on Animal Experimentation Project (NIH D-311 [C2]) covering limited findings currently available and subject to later confirmation or modification. Approximately 50 transplantation operations, predominantly on the rhesus monkey, have been performed—the majority autologous, a minority homologous, a small minority heterologous. Most transplants have been developing third molars, a minor group being single-rooted teeth; all were relocated in dental sites. Four categories were included: (1) tooth germs, prior to development of formed enamel or dentin; (2) partially developed tooth germs, i.e., prior to root formation: (3) developing teeth with partially developed roots; and (4) fully developed and apically narrowed teeth. Duration of individual experiments varied from 1 month to 1½ years. This report relates to early findings in the second and third categories; final reports on all categories will be presented later. Evaluation at this point consisted, necessarily, of (a) roentgenographic and clinical findings alone, and (b) roentgenographic, clinical, and microscopic findings. Group III.—In approximately one half of autologous cases of this category, acceptable results were obtained as estimated roentgenographically. In others there was evidence of varying degrees of root resorption or of filling in of the pulp chamber with calcified material. In cases ready for microscopic study, evidence of extensive resorption and some ankylosis was observed, with one instance of an ingrowing "spike" of alveolar bone free in the pulp canal. Postoperative lining of pulp canal surfaces with "osteodentin" may subsequently be followed by deposition of tubular dentin. Tentative evidence of a higher degree of re-establishment of those cases with adjunctive cortisone treatment was noted. Homologous cases tended, after short postoperative periods, to be eliminated. Group II.—Tentative results of limited autologous cases tend to be in harmony with substantial human clinical experience, in that transplantations of partially developed tooth germs, lacking roentgenographic evidence of beginning root formation, have developed inadequately, with extensive resorption within 6 months. Limited homologous experiments in the monkey or the cat tend to loss of transplant, as does heterologous transplantation (cat to monkey). Subsequent reports will cover all categories with more comprehensive microscopic findings, results of longer test periods and with comparative effects of biologic adjunctives.

9b. Zone Electrophoresis of Saliva.—*Irwin D. Mandel, and Solon A. Ellison School of Dental and Oral Surgery, and College of Physicians and Surgeons, Columbia University, N. Y.* In attempting to use paper electrophoresis in an investigation of human saliva, we found that the several published procedures gave irregular and contradictory results. Studies therefore were conducted to determine optimal conditions. The variables examined included methods of collecting, concentrating, and storing of the saliva, pH (pH4—pH11), buffer systems (veronal, phosphate, phosphate-citrate, phosphate-NaOH, acetate, citrate, borate, glycine-NaOH), current, time, temperature, and supporting media (various papers, agar, and glass curtains). The best results were obtained using whole saliva collected and centrifuged in the cold, lyophilized in siliconized flasks, and concentrated 10 to 20 times by redissolving in a suitable volume of 1M NaCl. This solution was then dialyzed against either pH 6.3 phosphate-citrate buffer 0.05 unit or pH 8.6 veronal buffer 0.05 unit and the same buffer used in the cell. Electrophoresis was

performed on S&S 2043A mgl. paper in a Spinco Model R Cell for 16 hours, at 10 to 15 Ma. per cell, in the cold (4° C.). When stained with bromophenol blue, 5 to 6 components were regularly and consistently distinguished using phosphate-citrate buffer and 3 to 4 with veronal buffer. The identity of these components is being investigated by chemical and immunochemical technics.

II. ORAL AND SALIVARY BACTERIOLOGY. MARCH 19, AFTERNOON, SECTION B.

10. EVALUATION OF A METHOD TO DETERMINE PRESENCE OF FOCUS OF INFECTION.—*Louis I. Grossman, School of Dentistry, University of Pennsylvania, Philadelphia.* There is no reliable method for determining the presence of a periapical focus of infection. It is known that a transient bacteremia will occur when periodontally involved teeth are traumatized, as by chewing hard candy, the microorganisms in the pocket being set free into the blood stream. The purpose of this study was to determine whether microorganisms, if present in the root canal, will be set free and produce a transient bacteremia when a tooth is traumatized. *Method.*—Untreated pulpless teeth with areas of rarefaction were used in this study. Seven milliliters of blood was removed from the median basilic vein of the arm, the tooth was traumatized for 1 minute with a Hollenback condenser fitted with a rubber tip, and 7 milliliters of blood was again removed from the arm immediately afterward. Aliquot amounts of the blood were placed in test tubes with and without "Liquoid," and the tubes were incubated at 37° C. Immediately after removal of the blood, the root canal was entered and 2 cultures were taken to determine whether the canal was infected. The cultures were incubated aerobically and anaerobically. *Results.*—In 16 cases, both preoperative and postoperative blood cultures were negative. Root canal cultures were positive in 8 of the 16 cases. In 21 additional cases, instrumentation of the root canal was carried out under usual clinical conditions, preceded and followed by blood samples for culturing. In one of the 21 cases, a diphtheroid bacillus was recovered both from the root canal and from the blood.

11. BACTERIOLOGIC STUDY OF INFECTED PRIMARY MOLARS.—*Sumner Joress, M. Michael Cohen, and Louis J. P. Calisti, Boston Dispensary, Boston and New England Medical Center Hospital, Brookline, Mass.* Fifty children with infected primary molars were selected for the purpose of studying the bacterial flora in open carious devitalized primary molars. The infected primary molars were isolated with a rubber dam and the field of operation sterilized with a 1:1000 Zephiran chloride solution. Cultures were taken of the pulp chamber with a sterile cotton point, and the cotton point was placed in a vial containing 1 c.c. of sterile normal saline solution. A control culture was taken from the gingiva of the opposite molar in the same jaw. The vial containing the saline solution and the contaminated paper points were inserted into a Kahn shaker and vibrated for 5 minutes. With a sterile platinum loop about 1 mm. in diameter, an aliquot of the contaminated saline solution was inoculated into the following media: (1) trypticase soy agar with 5 per cent sterile sheep blood; (2) eugon agar with 5 per cent sterile sheep blood; (3) brewer thioglycollate medium; and (4) tomato juice agar—(a) blood agar plates were incubated for 48 hr. at 35° C., (b) thioglycollate tubes were incubated for 5 days at 35° C., (c) tomato juice agar plates were incubated for 48 hr. at 25° C., and (d) aerobic and anaerobic subcultures were made from the thioglycollate medium wherever growth appeared. The subcultures were then correlated with the original streak plates. The organisms isolated, in

order of their predominance, were: (1) *Streptococcus salivarius,* (2) *Streptococcus mitis,* (3) micro-aerophilic streptococci, (4) beta hemolytic streptococci, (5) lactobacilli, (6) *Staphylococcus albus,* and (7) coliform bacilli (isolated from children with generalized chronic inflammatory gingivitis).

12. Isolation of Pleuropneumonia-Like Organisms From the Oral Cavity.—*I. L. Shklair and M. A. Mazzarella, Naval Dental Research Facility, Great Lakes.* Pleuropneumonia-like organisms (PPLO) are a filterable, highly pleomorphic and fastidious group of microorganisms. They are characterized by the lack of a rigid cell wall, extreme pleomorphism, and a distinctive colonial morphology. They have been isolated from individuals showing generalized inflammatory or infectious processes, but infrequently isolated and studied from the oral cavity. Both saliva and gingivae scrapings from young male naval personnel were cultured for PPLO to determine the best method of isolation. The specimen was inoculated into 5 ml. of Difco PPLO broth enriched with 10 per cent horse serum and 1 per cent yeast autolysate. In addition, thallium acetate (1:2000) and penicillin (3,000 units/ml.) were added to prevent bacterial overgrowth. The broth was incubated 36 to 48 hours at 37° C., and then 0.1 ml. was streaked on Difco blood agar base or Difco PPLO agar adjusted to a pH of 7 to 8, and enriched with the above-mentioned nutrients, but without the bacterial inhibitors. The Petri dishes and broths were incubated anaerobically (95 per cent nitrogen and 5 per cent carbon dioxide) for 48 hours and examined microscopically (150 ×). Atypical PPLO colonies that could not be subcultured were observed with aerobic technics, and the incidence of isolation was only 75 per cent. Using our anaerobic cultivation technic, the over-all incidence of recovery was increased to 83 per cent, typical PPLO colonies developed and were readily subcultured.

13. Prevalence and Clinical Significance of Pleuropneunomia-Like Organisms in the Oral Cavity.—*M. A. Mazzarella and I. L. Shklair, Dental Research Facility, Great Lakes.* The etiologic agent of bovine pleuropneumonia is the prototype of a distinct group of parasitic and saprophytic, filterable microorganisms which exhibit properties different from bacteria, rickettsiae, or viruses. Although they have been implicated in diseases of man and animals and are frequently found in the human rectal, genital, and oral tracts, their etiologic role is still obscure. The pathogenicity of oral strains is also undetermined, so relationship was sought to disturbances of the oral mucosa. The human oral species is notable for its inability to ferment refined carbohydrates and thus, in the present work, became the criterion for the selection of patients to determine if the presence of these organisms was associated with the caries-immune state. Young naval recruits of two types were used, caries-rampant and caries-immune. Oral examinations recorded the number and extent of carious lesions, extracted teeth, unerupted teeth, type of dental deposits, condition of the gingivae, and apparent health of the investing structure. The presence or absence of the *pleuropneumonia* group could not be correlated with any type of dental deposits or pathologic condition of the gingivae. Working at practical certainty range, a statistical trend was noted between the absence of PPLO and the caries-rampant state. Statistical relation was found between low DMF scores and the presence of PPLO. A statistical relationship was also noted between low *lactobacilli* counts and the presence of PPLO in the oral cavity.

14. Growth and Incidence of L and Pleuropneumonia-Like Organisms.—*Clarence P. Canby, Eleanor Gilmore, and George W. Burnett, Walter Reed Army Institute of Research, Washington, D. C.* A liquid medium and culture

technic were developed which would be optimal for the isolation and maintenance of oral L organisms of diverse bacterial origin without the presence of a specific transforming agent. This technic was also suitable for growth of oral pleuropneumonia-like organisms (PPLO). By this technic, material from the oral cavity was cultured in a composite veal-thioglycollate (V-T) broth for no longer than 96 hours, after which the L organisms were subcultured for two or three times on penicillin-containing media in order to stabilize them sufficiently to grow indefinitely in liquid media containing no transforming agent. Utilizing this culture technic, the incidence of both PPLO and L organisms was determined in unstimulated saliva, scrapings from teeth, and carious material of human beings. Eighty-three per cent of 52 salivas from different individuals were found to contain PPLO and L organisms; 94 per cent of the tooth scrapings were positive while 14 per cent of the material from carious lesions was positive for the organisms. In all individuals examined, either the tooth scrapings or saliva were positive for either PPLO or L organisms. In general, L organisms were more prevalent than PPLO.

15. CONTINUED STUDIES ON AN ORAL GRAM-POSITIVE FILAMENTOUS ORGANISM.—*Patricia H. Beck and Marion N. Gilmour, Eastman Dental Dispensary, Rochester.* Studies were continued on 56 strains of an oral gram-positive organism characteristically having a bacillus-like body attached to a filament. To test the effect of pH on growth, broth and agar brain heart infusion media were adjusted to a pH ranging from 4.5 to 9.0, and inoculated. Growth occurred between pH 6.0 and 8.5. All strains are true facultatives; under both aerobic and strictly anaerobic conditions, serial plate subcultures and broth growth from inocula of 2 cells per milliliter were successful, but dependent upon the medium employed. The biochemical tests previously reported were repeated aerobically after 6 months and were also performed anaerobically. All strains continued to ferment glucose, fructose, sucrose, mannose and maltose, and none produced acid or gas on lactose, glycerol, sorbitol, dulcital, inulin, rhamnose, arabinose, xylose, alpha-methyl-glucoside, melibiose, cellobiose, or starch. Any changes noted occurred on salicin or raffinose and could be attributed to late fermentations or poor growth. Aerobic fermentation tests resulted in a greater pH decrease with fermentable carbohydrates and a greater pH increase in the presence of nonfermentable substrates than did anaerobically incubated tests. All strains were nitrate positive, catalase positive, acetoin positive, produced CO_2 hydrolyzed starch, and were indole negative. The majority of the strains are very sensitive to penicillin, streptomycin, erythromycin, carbomycin, and bacitracin.

16. MODE OF REPRODUCTION OF AN ORAL GRAM-POSITIVE FILAMENTOUS ORGANISM.—*Basil G. Bibby and Marion N. Gilmour, Eastman Dental Dispensary, Rochester.* Sixty strains of an organism morphologically similar to Bulleid's *Leptothrix buccalis,* Bibby's *Leptotrichia buccalis,* and Morris's Leptotrichia were isolated. The predominating cell morphology in smears was a filament, 10 to 100 μ long and 1 to 2 μ wide, attached to a bacillus-like body twice its width. Time lapse photography of single cell cultures indicated the mode of reproduction was as follows: The filaments increased in diameter, formed cell walls at intervals, and fragmented to form a zigzagged row of bacillus-like bodies which varied in length. Each bacillus-like body formed one or more protrusions which shortly after formation were separated from the bacillus-like body by a cell wall. The protrusions elongated to form filaments which reproduced by the cycle described. Dichotomous branching was not seen in young cultures.

However, it did occur under some cultural conditions, the cells then resembling a Y. The Y forms reproduced by the fragmentation cycle described above. Free coccoid elements were not found either in microcultures or smears. The taxonomic positioning of this organism will be discussed on the basis of these findings.

17. Characterization and Streptococcal Antagonism of an Oral Filamentous Microorganism.—*Robert L. Richardson and Jean Schmidt, College of Medicine, State University of Iowa, Iowa City.* From 27 specimens of plaque and carious material, 10 strains of a microorganism similar to Bulleid's *Leptothrix buccalis* were isolated using the method of Gilmour and Hunter (J. Bact. 76: 294, 1958). Although the main morphologic structures were nonsegmented, gram-positive filaments, the unique and distinctive structures were whip-like forms which consisted of a bacillary element united to one end of a filament. The organism was considered not to be acid fast, although acid-fast granules were demonstrated in some filaments. Dichotomous branching was observed in most strains. Growth occurred either aerobically or anaerobically, and all strains were catalase positive, Voges-Proskauer positive, hydrolyzed starch, reduced nitrate to nitrite, and fermented glucose (no gas), sucrose, maltose, mannose, dextrin, and salicin. Glucose broths varied between pH 4.9 and 5.5 after 14 days' incubation. On a medium containing brain heart infusion agar, glucose, and yeast extract, growth of the filamentous organisms was inhibited by a 1:1000 dilution of unfiltered saliva; Seitz filtered saliva was not inhibitory. The inhibition was shown to result from a bacterial antagonism; growth of *Streptococcus salivarius, Streptococcus mitis,* and *Staphylococcus epidermidis* inhibited growth of the filamentous organism. Acid production by the streptococci and staphylococci, not hydrogen peroxide production, caused the inhibition.

18. Growth Studies of Oral Filamentous Microorganisms.—*George J. Kasai, Walter G. Zoller Memorial Dental Clinic, and University of Chicago, Chicago.* Several strains of oral filamentous nonbranching microorganisms were isolated from patients in the University clinics and laboratory personnel. Thirty-four strains were isolated from 20 different individuals. No attempt was made to correlate the oral conditions with the microorganisms isolated. In this series of cultures there appeared a spectrum of microorganisms as shown by their biochemical reactions and physical appearances. Studies made on the group indicated that they were anaerobic or microaerophilic. When grown on the surfaces of agar plates many of the strains failed to grow or grew to a very limited extent whenever the methylene blue indicator showed even a pale blue color. Few strains grew even under these conditions, indicating that they were microaerophilic in their requirements. CO_2 was not essential but stimulatory; tubes incubated with CO_2 gave better growth than those incubated under identical conditions, but with N_2 in place of CO_2. Studies were also made of their ability to grow at different temperatures. Most of them proved to be gram positive, some were intermediate, and some gram negative. A few strains had gram-negative sheaths with gram-positive granules and clumps within these sheaths. These strains also exhibited irregular forms, such as clubs and swellings in the middle of a long rod or filament, and other shapes.

19. The Substitution of Known Compounds for Ascitic Fluid in the Cultivation of Borrelia Vincentii.—*Edward G. Hampp and Thomas A. Nevin, National Institute of Dental Research, U. S. Department of Health, Education and Welfare, Bethesda.* The isolation and cultivation of the various

members of the family Treponemataceae on artificial culture media have been complicated, first, by the need for an adequate base medium and, second, by the absolute requirement of the microorganisms for ascitic fluid or blood serum products. The lack of knowledge concerning the nature of the indispensable factors present in these complex biologic materials has been a deterrent in the elucidation of the nutritional requirements of this little understood group of organisms. This study was concerned with the partial definition of the nutrition of a strain of *Borrelia vincentii* which permitted the cultivation of this organism in an infusion medium without natural enrichment. The basic medium employed in this work was sterilized in the autoclave at 121° C. for 15 minutes rather than by tyndallization, and growth of the spirochetes never developed to any extent in the absence of additives. Preliminary experiments with ascitic fluid revealed fairly high concentration of organic phosphates; a variety of coenzymes and phosphorylated intermediates were therefore screened in an attempt to replace the usual ascitic fluid enrichment. It was found that a mixture of five coenzymes (cocarboxylase, codecarboxylase, coenzyme A, adenosine triphosphate, and diphosphopyridine nucleotide) and glucose-l-phosphate would substitute for the ascitic fluid supplement normally required in the basic medium for the growth of *B. vincentii*. The organisms reached maximal growth (80-90 $\times$ 10^6 cells per milliliter) through 12 successive transfers in this medium. Furthermore, four strains of oral treponemes and three additional strains of *B. vincentii* reached maximal growth through two transfers in the same medium; however, three strains of *Borrelia buccalis* did not survive under these conditions.

20. STUDIES ON ANTIBACTERIAL ACTION OF HUMAN SALIVA: A LACTOBACILLUS BACTERICIDIN.—*Bernard J. Zeldow, University of Washington, Seattle.*—Further characterization of a salivary bactericidin active against *Lactobacillus acidophilus* ATCC 4357 is presented. This factor was quantitated in pure parotid and submaxillary salivas collected from 25 individuals. It was not detected in any of the corresponding serums. The component in the salivary secretions of the two glands appeared to be similar if not identical. It was observed that the bactericidal activity of the salivas disappeared upon dialysis against de-ionized water. The dialysate also lacked activity even after concentration by lyophilization. If, however, equal volumes of the dialyzed saliva and the concentrated dialysate were recombined, the original bactericidal potency was re-established. Whereas the fraction retained by the membrane was completely inactivated at 75° C. for 5 minutes at pH 7.0 to 10.0, it remained stable by such treatment at pH 5.5. The dialysate was found to remain stable under these conditions. The dialyzable component was not fully characterized. It is highly improbable that the active components were of bacterial origin. The evidence also indicated that it was not lysozyme.

21. EFFECT OF CANDIDA ALBICANS ON GROWTH OF LACTOBACILLI.—*Stewart A. Koser, Eleanor Hodges, Ilse Tribby, and Joan T. Stuedell, Zoller Dental Clinic, and University of Chicago, Chicago.* This investigation was concerned with one aspect of the problem of mutual relationships of oral microorganisms: influence of one species on growth of another. The effect of *Candida albicans* on growth of lactobacilli was studied under conditions such that the lactobacilli, without the yeast, were unable to multiply because of vitamin deficiencies of the basal medium. Since *C. albicans* needs only biotin, satisfactory growth of the yeast could be obtained in a medium deficient in several vitamins required by lactobacilli. During growth of Candida, other vitamins were synthesized by the yeast. Several strains of each of *Lactobacillus casei* and *L. plantarum*, after introduction of small inocula, often attained numbers of 50 to 100 million

per milliliter, with occasional counts of 400 to 500 million in the presence of the growing yeast. Without the yeast, little multiplication of the lactobacilli took place. *L. fermenti* also grew well with the yeast when reducing agents and pentoses were supplied. The pH levels reached in the course of associated growth of a lactobacillus and *C. albicans* were in most cases less acid than those attained by equivalent numbers of the same lactobacillus alone. In contrast to results with the above three species of lactobacilli, *L. acidophilus,* which is more exacting in its growth requirements, either failed to grow or grew only to a limited extent in association with *C. albicans,* even after the addition of vitamin B_{12}.

III. Clinical Studies. March 19, Afternoon, Section C.

22. Variation of Integumental Landmarks in Relation to Lip Posture. —*Charles J. Burstone and Warren Hamula, Indiana University, Indianapolis.* A sample of 32 adolescents were selected on the basis of facial excellence. A method of analysis was developed in order to study changes in the position of integumental landmarks as associated with lip posture. Two primary lip positions were established with the mandible in rest position. In one the lips were parted and relaxed and in the other they were lightly closed. Measurements were made from labial headplates, employing the Broadbent technic, exposed to demonstrate both hard and soft tissues. The labial gap (amount of vertical lip closure) averaged 3.7 mm. In a horizontal plane, integumental landmarks from subnasale to menton did not change appreciably from the relaxed to the closed position. In the vertical plane, change of position was noted with the descent of superior labial sulcus (1.1 mm.). Landmarks in the lower lip moved superiorly from the relaxed to the closed position; labrale superius 2.5 mm., inferior labial sulcus 2.1 mm., and menton 2.6 mm. On the basis of these findings standards were established which may have value as a guide to muscular imbalances of the orbicularis oris complex.

23. Periodontal Aspects of Mongolisim.—*Richard A. Winer and M. Michael Cohen, Tufts University, School of Dental Medicine, and Fernald State School, Waverly, Mass.* One hundred mongoloid patients, ranging in age from infancy through 20 years, were selected for this study. Under standardized conditions, color intra-oral Kodachrome photographs were taken on each patient. A careful clinical examination of occlusal relationships of jaws, the size and number of teeth present, gingival and periodontal disturbances were recorded on specially designed I.B.M. cards. Intra-oral radiographs were also taken. Other anomalies (eyes, ears, etc.) found in these patients were recorded on another I.B.M. card. Periodontal disturbances were observed in the primary dentition in mongoloids between the ages of 3 and 5. Generalized chronic, inflammatory, gingival enlargement was present in the majority of these children. Calculus and gingival recession, as well as pocket formation, were also observed. Several patients in this age group had a premature exfoliation of the mandibular incisors and molars. The most interesting finding in approximately 50 per cent of the cases was a midline defect in the mandibular incisor region. Many of the mandibular permanent incisors had been exfoliated in patients between the ages of 11 and 14. When the mandibular incisors were present, bone loss (periodontosis) was severe and with marked mobility in young mongoloids 9 through 13 years of age.

24. MEASUREMENT OF INTRUSION OF THE UPPER INCISOR TEETH WITH FORCES BELOW 1,000 GRAMS.—*Gilbert J. Parfitt, University of Alabama School of Dentistry, Birmingham.* An instrument using transducer units was used. One unit recorded the movement of the tooth which was pressed upon and another recorded the force applied. The prototype of this instrument has been described (J. D. Res. 37: 64-65, 1958). Measurements of tooth movement were made from an adjacent reference tooth which was stabilized in the socket by a load of 100 grams. Application and removal of forces external to the tooth being measured did not affect the static force on the reference tooth. Measurements were accurate to 0.0005 mm. With application and removal of force, an initial intrusive displacement took place from which recovery occurred slowly. This suggested an initial displacement of fluid. Greater force was resisted and slight displacement with almost instantaneous recovery on removal of force suggested an elastic material to be involved. Displacement, however, was not proportional to the force applied, nor was the position of this support stationary within the socket, but became raised or lowered according to the duration and amount of force applied immediately prior to measurement. In one series of tests, the initial displacement varied between 0.003 mm. and 0.012 mm.; secondary displacement with 500 grams varied between 0.008 mm. and 0.014 mm. Displacement between 200 and 1,000 grams was 0.003 mm. to 0.006 mm. and between 10 and 200 grams varied between 0.006 and 0.012 mm.

25. THE PREVALENCE OF MALOCCLUSION IN 491 FOUR-YEAR-OLD CHILDREN.—*Louis J. P. Calisti, M. Michael Cohen, and Martha H. Fales, Brookline Health Department, and Tufts University School of Dental Medicine.* In May of 1958 in Brookline, Massachusetts, an orthodontic survey was carried out on 491 preschool children with a mean age of 4.65 years who were to enter school in September of 1958. The criterion used for assessing the occlusal relationship of the dental arches was based on Angles' classification of malocclusion (neutro-occlusion, disto-occlusion, and mesio-occlusion) with the teeth in centric relation. The following is a distribution of malocclusion found in the 491 subjects: neutro-occlusion, 473 (96.3 per cent) children (Note: 17 children in this group manifested unilateral and bilateral posterior crossbite); disto-occlusion, 13 children (2.7 per cent); and mesio-occlusion, 5 children (1.0 per cent). Contrary to prevailing opinion of even spacing of the incisors in the primary dentition, 349 subjects (71.1 per cent) showed no spaces in the maxillary and mandibular incisor regions. One hundred and one (20.5 per cent) of the 491 children indulged in oral habits. Seventy-five (74.3 per cent) children with oral habits demonstrated varying degrees of open bite while 26 (24.6 per cent) children showed no effect on occlusal relationship. The distribution of malocclusion according to socioeconomic level was as follows: low income—among 230 children, 16 (6.9 per cent) had malocclusion; middle income—among 161 children, 13 (8.1 per cent) had malocclusion; and high income—among 100 children, 6 (6.0 per cent) had malocclusion. This method of assessing malocclusion has been found simple for recording epidemological data on the prevalence of malocclusion of the primary dentition.

26. THE EVANSTON DENTAL CARIES STUDY. XIX. PREVALENCE OF MALOCCLUSION OF CHILDREN IN A FLUORIDATED AND CONTROL AREA.—*Iden N. Hill, James R. Blayney, and Walter Wolf, University of Chicago, Chicago.* Three groups of children, (a) the Evanston fluoride-free base line group, (b) the Evanston children with 8 to 10 years' exposure to fluoridated water, and (c) the fluoride-free control group, all 6 to 8 and 12 to 14 years of age, were

selected to determine the prevalence of malocclusion. The classification of malocclusion was judged primarily from the position mesiodistally assumed by the first permanent molars on their eruption and locking. The cases were classified as to type of occlusion, orthodontic treatment, and cases with and without premature loss of one or more second deciduous molar teeth. The Evanston children in the fluoride group had a greater percentage of cases of normal occlusion than the Evanston prefluoride base line group. The control group had slightly fewer cases in the normal classification in 1956 than in 1947 for all ages except the 14-year-olds. Of eighty-four children with a loss of one or more second deciduous molar teeth prior to the first examination at 6, 7, or 8 years of age, 40 showed normal occlusion, and 44 showed malocclusion on subsequent examination at 12, 13, or 14 years of age. These particular children had no history of orthodontic treatment or the advantage of space maintainers.

27. Oral Environmental Factors in Human Twins.—*Sidney B. Finn, Robert C. Caldwell, and Mary H. Grogan, University of Alabama School of Dentistry, Birmingham.* Clinical and x-ray examinations were conducted and study models prepared on thirty-four sets of identical and thirty-one sets of fraternal twins of school age, containing both male and female pairs. The identical twins had an average DMF tooth difference of 1.35 per twin, and a difference of 2.29 DMF surfaces. The fraternal twins had an average DMF tooth difference of 1.74, and a DMF surface difference of 3.74. The identical twins had a df (deciduous) difference of 0.91 teeth and 2.62 df surfaces as compared to a difference of 1.84 df teeth, and 4.29 df tooth surfaces among the fraternal twins. There was an average difference of 1.29 permanent teeth and 0.85 deciduous teeth among the identical twins, and a difference of 1.90 permanent teeth and 2.06 deciduous teeth among the fraternal twins. These differences correlated with the caries susceptibility tests (Snyder), masticatory performance tests, and the widths of the interproximal spaces.

28. Heritability of Dental Caries and Some Related Salivary Components.—*Harold O. Goodman, Bowman Gray School of Medicine, Winston-Salem, N. C., John E. Luke, Samuel Rosen, and Emanuel Hackel, Michigan State University, East Lansing.* The purpose of the study was to determine whether or not hereditary factors influence dental caries and certain salivary components thought by some to be related to caries. Subjects for this study were 38 monozygotic and dizygotic twin pairs of high school and college age. Dental caries were determined by explorer and mirror examinations coupled with full-mouth x-rays. The salivary components measured included rate of flow, pH, and amylase activity. Parallel measurements were made on parotid salivas and on a mixture of sublingual and submaxillary secretions. Oral microflora were measured using media for miscellaneous bacteria, streptococci, and lactobacilli. Comparisons of mean intrapair variances calculated for monozygotic and dizygotic twins indicated that all variables measured, except lactobacilli counts, were regulated to a considerable extent by genetic factors in the twin sample studied. Further study of the relationship of the salivary components measured and caries frequencies in the twins are in progress.

29. Preliminary Report on a Technic for Studying Mandibular Motion.—*Harry Lundeen, B. S. Savara, Ellis B. Jump, University of Oregon Dental School, and W. L. Parker, Reed College, Portland.* The technic developed uses multiple-exposure photography of landmarks fixed with respect to

the mandible and the upper face. These landmarks are 1 mm. ball bearings attached to external aluminum trusses rigidly fastened to the mandibular and maxillary teeth by cast Vitallium dental splints covering the buccal and lingual surfaces without occlusal interference. Two landmarks, approximately 16 mm. apart on the fixed maxillary truss, serve as controls. The three mandibular landmarks are placed: (1) at the center of the condyle, (2) in the lower bicuspid region, and (3) approximately midway between the other two. A General Radio "Strobolume" and "Strobotac" operated at ten flashes per second give an intense light source of small area. By exposing for one second, 10 positions are photographed. Glass photographic plates are used to avoid any distortion in the negatives. Since only the highlight is photographed, the point appears on the negative as a circle about 0.05 mm. in diameter. Two images can be resolved if their centers are separated by 0.025 mm. (Rayleigh criterion) which with the photographic reduction of about 4× corresponds to 0.1 mm. motion of the corresponding point on the mandible. The error, when a coordinate comparator is used to measure the position of the images, is less than one third of the resolution. From such measurements, the angle of rotation of the mandible and the actual motion of any point other than those measured can be calculated. Preliminary investigations using the technic show promise.

30. A NEW RADIOGRAPHIC TECHNIC FOR DIAGNOSING TEMPOROMANDIBULAR JOINT DISORDERS.—*J. B. McCabe, S. E. Keller, and B. C. Moffett, School of Dentistry, University of Alabama Medical Center, Birmingham.* A study of pathologic temporomandibular joints in human skulls showed the following gross changes: increased porosity and roughening of the subchondral bone, marginal exostoses, and bony lipping at the attachments of the joint capsule. These changes appeared most frequently on the posterior slope of the articular tubercle, especially on the lateral half, and were sometimes seen on the lateral half of the condyle. Such alterations were difficult to identify in a lateral radiograph. An x-ray technic was developed, therefore, which would show the entire lateromedial extent of the crest of the articular tubercle and the condyle. This was accomplished by using an oblique infraorbital view. The x-ray machine was fitted with a metal cylinder about 1 inch in diameter and 6 inches long to reduce the secondary radiation. The central ray was directed from a point inferior to the opposite orbit, through the maxillary sinus, nasal cavity, and infratemporal fossa, to the condyle. This projected the joint lateral to the mastoid process and reduced bony superimposition to a minimum. A head positioner and film holder were designed to help align the x-ray machine, patient, and film. The exposure was made on no-screen film at 65 kv. and 10 Ma. for 2 seconds. By using this technic on dissecting room cadavers, several pathologic temporomandibular joints were identified radiologically and verified later by dissection. A clinical study on patients with temporomandibular joint disorders showed that the oblique infraorbital radiograph was of greater diagnostic value than the lateral radiograph.

31. PRELIMINARY STUDIES ON MANDIBULAR DEFORMATIONS AND FRACTURES.—*Donald F. Huelke, University of Michigan Medical School, Ann Arbor.* In a highly selected group of 27 human mandibles, the location of areas of tensile strain was determined by the use of a strain-sensitive lacquer (Stresscoat). Under sufficient load, the lacquer cracks in response to the underlying tensile strain in the bones on which it has been sprayed. Static loadings (25 to 70 lbs.) were applied to the mid-sagittal chin point of edentulous and dentulous specimens, and those with only the anterior teeth. The condyles rested in

nonmovable cupped base plates. Deformation patterns showed that tensile strain first developed in the lateral subcondylar regions and on the labial or buccal surface of alveoli, indicating an inward bending and twisting of the condyles, and a mesiodistal compression, with outward bending, of the lateral wall of the alveoli. Fixing one condyle and permitting the other to slide freely, tensile strain patterns appeared in the subcondylar region on the medial and lateral sides, respectively. Secondary strain patterns indicated a stretching of the bone about the mental foramina and a flattening of the chin. Additional tension patterns about areas farthest away from the neutral axis or areas with marked irregularities—lingula and mandibular foramen, lingual tubercle—indicated that these are regions of high stress concentration. Differences in gonial angulation, state of dentition, or marked ruggedness of the bones had little influence on the location of the major deformation patterns under these conditions of loading and orientation.

32. Effects of Artificial Tooth Form on Bone Deformation Patterns.—*John J. Sharry, Harold C. Askew, University of Alabama School of Dentistry, Birmingham, and Heinz Hoyer and A. K. Heidberg, Hamburg, Germany.* In order to ascertain the effects of occlusal forms on the edentulous maxilla and mandible, a technic described by Gurdjian and Lissner for studying deformation patterns in bone was adapted to this problem. Duplicate sets of dentures were built for a series of edentulous skulls. These dentures differed only in the fact that one had 0° tooth form and the other a 30° form. Two to three millimeters of a soft plastic material was interposed between denture base and bone to simulate the function of the mucosa. The skulls were coated with a strain-sensitive lacquer and mounted on an apparatus designed to simulate centric, right and left lateral and protrusive movements. An aggregate force of 6 Kg. was chosen because it is well below the accepted averages for forces used by full denture wearers. If the bone is deformed, this lacquer will crack in a definite pattern. Testing for patterns was performed after each excursion and photographically recorded for comparison. The patterns obtained in dry bone specimen are applicable to the living situation. A total of eighteen skulls were used in the experiment. Results indicated that nonanatomic tooth forms balanced with a ramp result in minimal stress patterns while anatomic tooth forms properly balanced cause much more bone deformation.

33. The Forensic Use of Saliva.—*Frederick W. Kraus and Stitaya Sirisinha, VA Hospital and University of Alabama School of Dentistry, Birmingham.* Saliva has been the object of medicolegal investigations only to the extent of human blood group determinations. The identification of saliva as to its species origin has not been reported. This study attempted to demonstrate, by means of a capillary precipitin technic, the presence of species-specific substances in whole saliva and salivary gland secretions. A rabbit antiserum prepared commercially (Cappel) against whole human blood reacted with the following antigens: human serum (diluted up to 6,500 times), human saliva (10 to 40 times), rhesus serum (4,000 to 6,000 times), rhesus saliva (5 to 10 times), dog serum (10 to 100 times), and horse serum (1 to 10 times). Nonspecific positive reactions were eliminated by normal serum controls, and cross-reactions were abolished by antigen dilution or by absorption. The precipitin reaction remained demonstrable after boiling, freezing and thawing, filtration, and drying of saliva. Deproteinization abolished it. The tests were positive regardless of the presence of ABO substances in saliva, and the A and B blood group antigens did not react with the antihuman serum.

IV. EMBRYOLOGY AND DENTAL HISTOLOGY. MARCH 20, FORENOON, SECTION A.

34. PRENATAL INFLUENCES ON FETAL DEVELOPMENT.—*Seymour J. Kreshover and Lee E. Feild, National Institute of Dental Research, U. S. Department of Health, Education, and Welfare, Bethesda.* A condition of anoxia or of external pressure on a growing fetus might have specific application to the mechanism of embryological defects in human beings. To explore the latter question, 79 gravid rats, ranging from 13 to 16 days in gestation, had their uteri exposed and a fine needle puncture made through the amniotic sac of each embryo contained in one horn. The nonpunctured horns served as controls. Uterine contents (461 experimental and 289 control fetuses) were examined on the twentieth gestational day. The incidence of experimental fetal resorption was 7 per cent in rats operated at 16 days' gestation, 28 per cent at 15 days, 60 per cent at 14 days, and 38 per cent at 13 days. Examination for incidence of congenital defects showed palatal clefts and limb deformities (malrotation, agenesis, and syndactyly) in 0, 5, 33, and 6 per cent, respectively, of the offspring of rats operated at 13, 14, 15, and 16 days of gestation. In the study to evaluate anoxia, 124 gravid rats (6 to 15 days in gestation) had their right or left uterine and uteric arteries occluded for periods ranging from 10 to 120 minutes. The opposite horns served as controls. Examination of cesarean-delivered young (726 experimental and 440 control) showed the incidence of experimental fetal resorption to be 31 to 66 per cent in rats operated on the fourteenth to fifteenth days of gestation, 50 to 80 per cent in 12- to 13-day gestational animals, and 13 to 46 per cent in rats treated during the first trimester. A 2.5 to 5 per cent incidence of cleft palate and limb deformities was without apparent relationship to either duration of circulatory arrest or period of gestation when occlusion occurred.

35. INHIBITION OF SKELETAL FORMATION IN CHICK EMBRYOS FOLLOWING ADMINISTRATION OF TETRACYCLINE.—*G. Bevelander, H. Nakahara, and G. Rolle, College of Dentistry, New York University.* It has been shown previously that tetracyclines have an affinity for developing skeletal elements, and also that these substances are retained in the skeleton for some time. The present study dealt with the effect produced on skeletal development by the introduction of tetracycline to developing chick embryos. Two and five tenths milligrams of this substance was injected into the yolk sac of fertile eggs on the eighth day of incubation. Embryos were removed on the tenth, twelfth, fourteenth, and sixteenth days of incubation. Examination of the embryos showed a marked reduction in growth after the tenth day. There was also a corresponding reduction in the length, as well, as a marked bowing of the bones. Microscopic examination of these bones revealed that cartilage resorption did not occur in the normal manner. Also observed was an increase in the normal amount of uncalcified trabeculae and a corresponding decrease in the calcified trabeculae. Examination of these bone sections in ultraviolet light revealed the presence of a fluorophore, which indicated that tetracycline or a derivative was incorporated and retained in the calcified part of the bones formed during the time the embryos were subjected to the experimental environment. Under the conditions of our experiments tetracycline inhibited skeletal formation.

36. HEREDITARY VARIATION IN THE CRANIOFACIAL SKELETON.—*Sidney L. Horowitz, Richard H. Osborne, and Frances V. DeGeorge, Columbia University, School of Dental and Oral Surgery, and Institute for the Study of Human Variation, New York.* The relative importance of genetic variation in the anteroposterior and superior-inferior dimensions of the craniofacial skeleton was

studied by means of measurements obtained from the lateral cephalometric roentgenograms of twin subjects. Fifty-six like-sexed pairs of twins (35 monozygotic, 21 dizygotic) with a median age of 24 years comprised the study sample. The diagnosis of zygosity was based on serologic and morphologic criteria. Linear measurements (to the nearest 0.5 mm.) were recorded from tracings of the headplates as follows: face height (nasion-gnathion), anterior cranial base (nasion-sella), posterior cranial base (sella-Bolton point), ramus height (articulare-gonion), and mandibular length. Comparisons of the average intrapair variances of monozygotic and dizygotic twin pairs were carried out by means of Fisher's F test. Of the linear measurements analyzed, highly significant intrapair differences occurred between monozygotic and dizygotic twins in face height, anterior cranial base length, and mandibular length ($P = <0.001$ for all 3 cases), indicating a relatively high genetic component of variation in these dimensions.

37. Effects of Intrauterine X-Radiation on Bones and Teeth of Mice*.—*J. Lester Matthews and John A. Cameron, Baylor University College of Dentistry, Dallas.* Gravid albino mice at 15 days' gestation were divided into control and experimental groups. Mice of both groups were anesthetized and the gravid uteri were surgically exposed. Uteri of the experimental group were exposed to 300r in air at a target distance of 10 inches. Uteri of control animals were exposed to air for comparable periods of time. Following exposure, the uteri were repositioned and the wound closed. Birth times of litters were noted and experimental and control animals were sacrificed at intervals of 1, 7, 14, 21, 30, and 60 days. The heads were prepared for histologic study and the femurs were dissected free and sectioned longitudinally for determination of dimensions. Formalin fixation of heads was followed by clearing and ionic decalcification. Serial sections were prepared and the stage of tooth development, dentin apposition, and eruption rates were determined for the incisors and molars. Radiated animals demonstrated an initial decrease in rate of dentin apposition and slower eruption rate in all teeth, but teeth of radiated and control groups attained comparable size at 30 days postpartum. Radiation-delaying effects were least on the teeth of most advanced differentiation (incisors) at the time of radiation. Molars were similarly affected, with greatest effect on third molars and less effect on first molars. Femur dimensions were similarly reduced in radiated animals but approached those of control animals at the end of the 30-day period. These data demonstrate an initial inhibitory effect of radiation on rate of apposition and eruption but indicate a morphologic and dimensional recovery.

38. Homologous Transplantation of Tooth Buds.—*William Lefkowitz, College of Dentistry, The Ohio State University, Columbus.* Tooth buds offer desirable potentials in homotransplantation studies. The graft consists of embryonic cells which, after differentiation, form enamel and dentin. A take, or the rejection phenomenon may be examined in the graft, its product, or the surrounding host tissue. An inbred colony of Sherman albino rats provided the donor and host animals. All grafts were tooth buds secured from 19-day rat fetuses. Nine tooth buds were transplanted into fresh sockets of recently extracted first molars. None was recovered. Twenty-seven grafts were implanted beneath the dorsal skin and the submucosa anterior to the first molar. Thirteen specimens were recovered. They demonstrated the

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rejection phenomenon. Fifteen tooth buds were cultured in a medium containing host serum prior to implanting subcutaneously and submucously. Seven grafts were recovered. No rejection occurred. Grafts recovered up to 48 days after implantation demonstrated histocompatibility.

39. HISTOLOGIC CHANGES IN FIVE TRANSPLANTED HUMAN THIRD MOLARS.—*Ellis B. Jump, University of Oregon, Portland.* Microscopic examination of third molars transplanted to the alveoli of first molars and subsequently extracted for therapeutic reasons revealed the following changes: continued, postoperative growth of root; normal periodontal reattachment; metaplasia of pulp and odontoblasts to osteogenic and cementogenic tissues; postoperative apposition of osteodentin and cementum on previously-formed dentin; apparent, long-delayed partial recovery of pulpal cells to resume production of tubular dentin central to immediately postoperative osteodentin; and malformation of roots. The following sequence of events after the transplantation of the tooth germs is proposed to explain the above-mentioned histologic findings: (1) hemorrhage within dentinal papilla and follicular capsule at time of operation; (2) early organization of clot beneath capsule, leading to prompt recovery and continued growth of periodontium; (3) massive calcification of deeper clot within dentinal papilla following organization; (4) displacement into cervical region of pulp chamber of elements from capsule, which causes "pseudo periodontal" membrane to form on pulpal aspects of roots; (5) occasional recovery of embryonic pulpal tissue when operative trauma postoperative movements are minimal, resulting in resumption of tubular dentinogenesis upon older, reparative osteodentin; and (6) frequent displacement of sheath of Hertwig during transplantation, causing subsequent misshapen root formation.

40. THE USE OF A SEMILIQUID POSTOPERATIVE DIET FOLLOWING REIMPLANTATION OF HAMSTER SECOND MOLARS.—*Viola D. Flanagan and Hugh I. Myers, University of Kansas City, School of Dentistry.* In 1956, it was reported that reimplanted teeth in animals receiving a postoperative diet of unaltered Purina laboratory chow showed 65 per cent good takes, whereas animals that received the same diet made into a soft mash netted only 17 per cent good takes. Since the impaction of the soft mash into the extraction area was distinctly detrimental, no analysis of the possible effect of the hard food trauma was indicated. Studies were therefore initiated to test this factor. The preliminary experiments, using evaporated milk alone, produced diarrhea and death in a number of animals; therefore, a diet was used consisting of milk solids fortified with minerals and agar for added bulk. A group of animals having no surgery and fed this diet thrived and did not develop diarrhea. Reimplantation procedures were again resumed using the new postoperative diet. Thirty hamsters, 30 days old, having immediate replants were placed on the diet for 15 days postoperatively, then given unaltered Purina laboratory chow for 15 days, and sacrificed; 55 to 57 per cent of the teeth were good takes, or approximately the same as those having received the hard diet. This might indicate that the amount of possible trauma produced by the hard diet was not an overriding influence. It is also possible that the semiliquid diet was not as nutritionally ideal as the solid diet. Additional experimentation is being conducted in this connection.

41. RELATIONSHIP OF VASCULARITY ABOUT ERUPTING TOOTH GERMS TO RESORBING DECIDUOUS TEETH.—*D. Walter Cohen, Gerard J. Keller, and Morris Feder, University of Pennsylvania, School of Dentistry, Philadelphia.* Five

Sooty Manguebey monkeys with mixed dentitions were studied radiographically and microscopically. At time of sacrifice all animals were perfused with refined black India ink (carbon particles one μ or less) via the common carotid artery. The specimens were fixed in 10 per cent formalin for 48 hours. Undecalcified serial sections (100 μ to 1 mm. in thickness) of the maxillae and mandibles were cut and dehydrated in alcohol and cleared in a mixture of equal parts methyl benzoate and benzyl benzoate. The resorptive patterns of the roots of the deciduous teeth were studied. The space between the enamel epithelium of the permanent successor and the cementum and dentin of the deciduous tooth was occupied by a highly vascularized granulation tissue ("absorbent organ" of J. Tomes). The blood vessels of the vascular plexus in this tissue were well defined. These vessels were in close proximity to the enamel epithelium and the resorbing deciduous root, and numerous anastomoses were prominent. The number and thickness of the vessels about the succedaneous tooth germs were compared to those about permanent tooth germs without deciduous predecessors.

42. Noncellular Cementum of Human Teeth With Incomplete Apices.—*Carmen Umali Sabolboro and Harry E. Frisbie, University of California School of Dentistry, San Francisco.* Unerupted human third molars with incomplete apices were decalcified and sectioned for histologic study of noncellular cementum from its earliest stages of formation to completion of many lamellae. At early stages, groups of fiber bundles appeared perpendicularly to the dentin surface and later at the cementum surface as Sharpey's fibers. Cementoblasts appeared scattered among the fibers 2 to 5 μ from the surface. In addition, independent smaller fibers were seen interspersed between the larger Sharpey's fiber bundles. At all but the earliest stages many small fibrils swirled out from the larger bundles to assume irregular courses and even a direction essentially parallel to the surface. Within the formal cementum a similar basic orientation of fibers existed, but frequent twisting or bending from the plane of sectioning rendered it impossible to regularly follow the course of all the main and lesser fibers. Discontinuity of fibers with interposed homogeneous nonfibrous cementing bands was not encountered. A frequent bending out of small fibers from the larger bundles was observed, and it was apparent that 2 separate and independent fiber groupings for the Sharpey's fibers and the matrix fibrillae did not exist, but that these elements in toto formed the fibrous meshwork of the cementum. Silver staining offered evidence for irregularly disposed immaturities. Incremental bands occurred wherever these presented linear coincidence. The dentinoenamel junction was notable for its highly irregular contour, the lack of any definitive separating membrane and for the continuity or contiguity of the fibrillar bundles of the cementum and dentin.

43. Cementum Thickness of Teeth From India.—*Gerd Müller and Helmut A. Zander, Eastman Dental Dispensary, Rochester.* Cementum thickness was determined for 61 single-rooted teeth from India. These teeth were an unselected sample from the Hindu population; the only other known factor was the age of the patient at the time of extraction. They were divided into three age groups: 21-30, 31-40, and 41-50. The method for determining cementum thickness was the same as that previously described by Zander and Hürzeler. There was significantly less cementum on the roots of the "India teeth" than on the roots of the teeth in Zander and Hürzeler's study of healthy teeth obtained from Minnesota. The average thickness of the cementum from the "India teeth" was comparable to that of severely periodontally diseased teeth obtained from Minnesota.

43a. THE ROLES OF THE MAXILLA AND PREMAXILLA IN THE FORMATION OF THE FACE.—*Jay D. Decker and Bertram S. Kraus, School of Dentistry, University of Washington, Seattle.* There has been considerable debate in the literature concerning the ectofacial fusion of the maxilla and premaxilla. Chase, Inouye, and Noback and Moss support the thesis that the maxilla and premaxilla fuse ectofacially, without overlap of the latter by the former. Woo, on the other hand, claims that ectofacial fusion involves the overlapping of the entire premaxilla by the maxilla proper. It is the viewpoint of the present authors that neither thesis adequately explains the developmental situation. A series of 150 embryos, ranging in age from 7 to 18 weeks, was cleared and stained with alizarin red S. Each embryo was examined under a stereoscopic binocular microscope by palpation with microdissecting instruments, and from a number of specimens the entire maxilla was dissected out and examined from all aspects. Photographs and drawings were made of many specimens but it was found that neither could adequately illustrate the many-faceted developmental phenomena involved. It was found that macroscopically the bony structures of the frontal processes of the premaxilla and maxilla were distinct and remained so throughout the age period studied. The two processes fused along their entire lengths. Externally, however, both processes were overlapped by a trabecularized plate growing superiorally and medially from the labial aspect of the cuspid alveolus. Internally, secondary trabecularized plates of bone arose from both processes along their entire lengths. The internal plate of the premaxilla curved medially and then fused with the premaxillary process. That of the maxilla curved laterally and fused with the maxillary wall. Between the two a sulcus was formed. This sulcus remained quite distinct throughout the prenatal period studied.

V. CARIES: METALLIC IONS AND FLUORIDES. MARCH 20, FORENOON, SECTION B.

44. EFFECTS OF METALS ON RAT CARIES AND ENAMEL METAL LEVELS.—*L. C. Hendershot, Janet Forsaith, Biochemical Research Laboratory, and R. E. Mansell, Spectroscopy Research Laboratory, The Dow Chemical Company, Midland, Mich.* Zinc, nickel, or manganese (156 to 189 ppm) afforded a moderate protection against dental caries when added as the Versenate to the diet (50 per cent sucrose—50 per cent Purina) of female Wistar rats but not when added as the acetate. When combinations of Zn+Ni, Zn+Mn or Mn+Ni were used (total added cation: 329 to 362 ppm), the per cent protections for the latter combinations of metals fed as Versenates were 56, 14-39, and 65, respectively, and as the acetates, 29, 36, and 39, respectively. Combinations of Zn+Ni+Mn (total added cation: 516-518 ppm) resulted in protection in the Versenate group which was not significantly different from that in the acetate (43 per cent and 58 per cent, respectively). Before the teeth were examined for caries, about one third to one half the thickness of the molar enamel was removed with a diamond stone, the dust collected in a small electrostatic precipitator and analyzed for Zn, Ni and Mn by emission spectroscopy. Enamel levels of these metals in control animals on the cariogenic diet were as follows: Zn, 11-14 ppm; Ni, 2.6-5.0 ppm; and Mn, 37-60 ppm. In animals fed Versenate forms of Zn, Ni, or Mn, the enamel levels of Zn and Ni remained essentially unchanged while Mn increased slightly. In animals fed Zn, Ni or Mn acetates, Ni levels were essentially unchanged, Zn increased in Zn-fed animals to 56-85 ppm and Mn rose in Mn-fed animals to 185-275 ppm. The incidence of caries did not correlate with the levels of Zn, Ni, or Mn in the portions of enamel analyzed.

45. The Complementary Effect of Beryllium and Fluorine Upon Hamster Caries.—*Paul R. Thomassen and Henry M. Leicester, College of Physicians and Surgeons, San Francisco.* Two hundred and seventy-two male hamsters were on a diet containing 20 ppm of beryllium and fluorine for the period of molar calcification from conception to 30 days of postpartum life. These animals were then placed upon a cariogenic diet for 150 days. Upon sacrifice the molars were scored for dental caries under a 20-power microscope. There were 3,264 molar teeth available for study in the experimental group. Of these, 103 teeth were carious and gave a total occlusal score of 68.25. The average occlusal area score was 0.0209 ± 0.1367. The controls were made up of 233 male hamsters on a diet containing 20 ppm of fluorine for the like period of molar calcification. These animals were placed upon the same cariogenic diet for 150 days and then sacrificed. There were 2,796 molar teeth available for study in the control group. Of these, 140 were carious, having a total occlusal area score of 125.25 and a mean of 0.0448 ± 0.2427. The reduction of the occlusal area scores of the experimental group over the control was 53.3 per cent. A "t" test on the occlusal area scores of both groups of molar teeth gave a value of 4.852. This is statistically significant, giving a probability less than 1 per cent of being due to chance.

46. The Combined Anticariogenic Effect of a Single Topical Stannous Fluoride Solution and the Unsupervised Use of a Stannous Fluoride-Containing Dentifrice.—*Joseph C. Muhler, Indiana University, School of Dentistry, Indianapolis.* The clinical results using a single application of a 8 per cent solution of stannous fluoride and the promising anticariogenic effectiveness of a stannous fluoride—calcium pyrophosphate dentifrice containing stannous pyrophosphate, suggested to us that perhaps a combination of these two preventive dentistry technics might produce a greater reduction in dental caries than the use of the dentifrice alone. In order to clinically evaluate this hypothesis, a clinical study was designed in which 750 children, ages 6 to 15 years, were divided into 3 groups. Group I used a commercially available stannous fluoride dentifrice. Group II used the same stannous fluoride-containing dentifrice and, in addition, received a single application of an 8 per cent solution of stannous fluoride every 6 months. Each topical stannous fluoride application was preceded by a thorough oral prophylaxis. Group III received a dentifrice identical to that used by Groups I and II except that it contained no stannous fluoride or stannous pyrophosphate, and, in addition, received a single application of distilled water preceded by a thorough oral prophylaxis every 6 months. All of the children were divided into the three respective groups according to past caries experience, and each received a thorough prophylaxis, oral and radiographic examination each 6 months, the technics of which have been described previously. The results at the end of 6 and 12 months showed that Groups I and II had less dental caries than the control group. The group using the combination of a topical stannous fluoride application and a stannous fluoride dentifrice had a pronounced anticariogenic effect, amounting to a reduction of about 60 per cent at both the 6- and 12-month periods. The group using the dentifrice alone had a reduction of about 35 per cent. These data were highly significant statistically.

47. Effect of a Commercial Stannous Fluoride Dentifrice With Controlled Brushing on Caries in Children.—*George Peffley and Joseph C. Muhler, Indiana University, Bloomington.* In the originally conducted clinical tests which evaluated the effectiveness of a stannous fluoride–calcium pyrophosphate dentifrice, it was shown that the participating children brushed

their teeth on the average of less than once a day. While the results were encouraging it was evident that the oral hygiene habits were only average. Since regular use of the stannous fluoride dentifrice would be expected to produce better clinical results, a controlled brushing test was designed. This test was conducted in a military school where all the children liver under almost identical conditions. The age range of the subjects was from 9 to 18 years. All received a thorough clinical and x-ray examination at the beginning of the study and were divided into 2 groups upon the basis of past caries history. Group 1 received a stannous fluoride dentifrice and Group 2 the same dentifrice minus the stannous fluoride. All of the children were required to brush their teeth 3 times a day. Subsequent questioning showed that this was followed very closely. The dramatic improvement in their oral hygiene similarly indicated a definite improvement in their brushing habits. After 5 and 10 months the children were re-examined by the same dentist. All of the conditions of the study and the examinations themselves conformed to the rigid rules governing such tests as previously described and practiced. The results at both the 5-month and 10-month periods showed highly significant reductions when evaluated either by DMFT or DMFS indices. The results of this study would seem to suggest that a greater effectiveness of the stannous fluoride–calcium pyrophosphate dentifrice is associated with a more frequent use of the product.

48. SINGLE TOPICAL STANNOUS FLUORIDE APPLICATION: RESULTS AT END OF FOUR YEARS.—*Charles W. Gish, Charles L. Howell, Indiana State Board of Health, Indianapolis, and Joseph C. Muhler, School of Dentistry, Indiana University.* Four years ago approximately 500 school children, ages 7 to 8 years, were divided into two parts according to their past history of dental caries. Part 1 received an initial series of four applications of a 2 per cent sodium fluoride solution which was repeated after 3 years. The children in Part 2 received a single application of an 8 per cent solution of stannous fluoride each year. All fluoride applications were given immediately following a thorough dental prophylaxis except the second, third, and fourth NaF applications, which were given 2 to 7 days apart without a prophylaxis. The same dentist examined all the children. Each year new children in the second grade were added to the treatment series according to the previous description. At the present time 1,600 children are in the program. The data obtained on those children, started 4 years ago, indicate that the groups receiving the stannous fluoride each year had significantly less dental caries than the children receiving sodium fluoride when evaluated by DMFT and DMFS indices. The number of teeth erupting during the 4-year study period which became carious was significantly less in the children receiving stannous fluoride. Children started 1, 2, and 3 years ago on the treatment similarly show that the stannous fluoride was significantly more effective than the sodium fluoride in reducing the dental caries experience.

49. RELATIVE EFFICACY OF SODIUM N-LAUROYL SARCOSINATE IN REDUCING DENTAL CARIES.—*J. Hayden, College of Medical Evangelists, Loma Linda, Calif., and R. L. Glass, Tufts University, Boston.* A clinical study has been carried out to study the efficacy of sodium N-lauroyl sarcosinate in a dentifrice for the reduction of dental caries in the permanent teeth of school children. The participants were children attending a school in southern California. They were divided at random into two groups. The control group used a dentifrice containing 2 per cent sulfated, hydrogenated cocoanut oil monoglyceride as the detergent. The experimental group used the same base

dentifrice, but with sodium N-lauroyl sarcosinate as the detergent. At the end of a year, 332 subjects were available for re-examination; these were about equally divided between the two groups. The control and experimental groups showed the following mean values, respectively, at the time of the first examination: age, 11.8 and 11.6 years; sound teeth, 16.1 and 16.0; decayed and filled surfaces, 7.7 and 7.3. No significant difference was observed between the groups. Only interproximal caries were evaluated, this by posterior bite-wing and anterior vertical films. After an observation period of 1 year, the control group showed a mean increment of 1.09 decayed and filled surfaces; the experimental group, 0.67 decayed and filled surfaces. This difference was significant at the 2 per cent level.

50. In Vitro Study of the Effectiveness of Stannous Fluoride in Preventing Tooth Decalcification.—*Vincent Segreto and Norman Harris, School of Aviation Medicine, Randolph Air Force Base, Texas.* Studies were conducted with extracted noncarious teeth to determine the effectiveness of various concentrations of topically applied stannous fluoride in reducing the loss of both stable and radioactive phosphorus resulting from formic acid decalcification. There was an increase in protection with increasing concentrations of SnF_2, but the differences were most marked from 0 to 10 per cent with the increase from this point being only slight at the higher concentrations of 15, 20, and 25 per cent. Radionuclide studies indicated that the effect of sodium fluoride on P-32 exchanged teeth was different than with stannous fluoride. More P-32 was lost in decalcification following NaF application than with the water control. The use of SnF_2 resulted in approximately 80 per cent less loss of radioactive phosphorus than the control. The combination of successive applications of NaF and SnF_2 yielded no more protection than the latter alone. An equal mix of SnF_2 and pumice as a prophylaxis paste resulted in an approximate 50 to 60 per cent in vitro protection to formic acid decalcification. These results suggest that the use of a prophylaxis paste incorporating SnF_2, plus a subsequent topical application of a 10 per cent solution of SnF_2 should yield near maximum protection to acid decalcification.

51. A Comparison of the Increment of Fluoride in Enamel and the Reduction in Dental Caries Resulting From Topical Fluoride Applications.—*F. Brudevold, I. Sundvall-Hagland, W. D. Armstrong, D. E. Gardner, and F. A. Smith, Eastman Institut, Stockholm, Sweden, and Eastman Dental Dispensary, Rochester, University of Minnesota, Minneapolis, and University of Rochester, Rochester.* Deciduous teeth which exfoliated during and after a topical fluoride application study were collected and subjected to fluoride analysis. In the study one half of the dentitions of 107 children were treated 4 times with 2 per cent NaF. The untreated teeth and the teeth of a similar group of children receiving no treatments served as controls. The caries reductions after 1, 2, and 3 years were 19, 14, and 9 per cent, respectively, when based on a comparison of treated and untreated teeth in the same mouth, and 34, 23, and 12 per cent when teeth of untreated children were used as controls. Analysis of the exfoliated teeth showed that topical fluoride applications caused an increase of approximately 50 ppm in the fluoride concentrations of the outer portion of the enamel. There was no detectable loss of fluoride from the treated enamel during the period 3 to 7 years following the fluoride treatment.

52. Effect of Fluoride on Enamel and Dentin Solubility in Acetate and Aspartate Buffers.—*Michael G. Buonocore, Eastman Dental Dispensary,*

Rochester. In studying the effect of different ions on enamel and dentin solubility it was found that the presence of aspartate ions in an acid-decalcifying environment significantly reduced its ability to dissolve enamel but not dentin. Since enamel solubility-reducing effects of this sort may be important in caries initiation, the aspartate effect was further studied in the presence of fluoride which is known to render enamel less soluble. One hundred milligram samples of 200 to 270 mesh enamel and dentin were shaken for 30 minutes in 50 c.c. pH 4 of acetate and aspartate buffers containing fluoride ion. Filtrates were analyzed for P.

BUFFER	ENAMEL DISSOLVED AS mg.P						DENTIN DISSOLVED AS mg.P		
PPM fluoride in buffer	0	1	10	100	200	500	0	1	10
Acetate	7.3	5.0	3.5	2.9	3.5	3.5	8.7	8.9	7.6
Aspartate	3.4	2.4	2.5	2.1	2.7	2.7	8.7	8.5	7.5

Fluoride produced a similar, small solubility-reducing effect on dentin in both buffers. With enamel, progressive solubility decreases were observed in both buffers up to 100 ppm F. At 200 and 500 ppm, enamel solubility increased to level found at 10 ppm for both buffers, presumably due to CaF_2 formation. Differences in enamel dissolved by the buffers decreased up to 100 ppm F and thereafter remained constant. It required 10 ppm of F to reduce the enamel solubility in acetate to that found in aspartate without fluoride.

53. TRANSPORT OF F^{18} IN MATERNAL FETAL CIRCULATION OF THE RAT.—*M. Michael Cohen, Richard A. Winer, and William A. Curby, Rotch Research Laboratory, Boston Dispensary, Boston, and Massachusetts Institute of Technology, Cambridge, Mass.* Fluorine^{18} was prepared in the Cyclotron at the Massachusetts Institute of Technology. Solid Li_2CO_3 target material was admitted into a reaction flask and dissolved with 1:1 H_2SO_4, which was introduced from a separatory funnel. The reaction mixture was heated to 140° C. for about 5 minutes. The HF generated was swept out with a gentle stream of nitrogen and collected in a receiving bottle (pH 8 with NaOH). F^{18} was formed from the reaction O^{17}(d,n) F^{18} and O^{18}(d,2n) F^{18}. Fluorine^{18} had a half-life of 112 minutes and the simplest possible decay scheme, emitting only a single positron of 0.65 mev. energy. Its daughter was stable oxygen^{-18}. Previous studies on fluoride content of placental tissues as related to content of drinking water have been determined by chemical methods. In this study, F^{18} was injected intraperitoneally into dated pregnant rats to determine the amount of F^{18} distributed in maternal and fetal tissues. Some rats were sacrificed 1 and 2 hours after injection while 2 gestating rats had laparotomies performed and 2 fetuses removed from each by cesarean section. The rats survived the surgical procedure and delivered the remaining litter normally. Urine, blood, uteri, placentae, and fetuses were examined for F^{18} activity employing a thin window gas counter. The greatest F^{18} activity was found in the urine with measurable amounts of the isotope detected in the blood, placentae, uteri, and fetuses. F^{18} injected intraperitoneally into rats in the second and third trimester of gestation could always be detected in the fetuses 1 and 2 hours later, indicating placental permeability to fluorine.

54. HUMAN PLASMA FLUORIDE CONTENTS.—*W. D. Armstrong and Leon Singer, University of Minnesota, Minneapolis.* The communal waters of Rochester, Minn., Minneapolis-St. Paul, Minn., Gettysburg, S. D., and Lake Preston, S. D., contain 0.15, 1.1, 2.5, and 5.4 ppm fluoride, respectively. The

following mean results with standard deviations, expressed as ppm fluoride, were obtained on analyses of blood plasma of adults of at least 5 years local residence: Rochester, 0.14 ± 0.020 (16 persons); Minneapolis-St. Paul, 0.14 ± 0.019 (25 persons); Gettysburg, 0.17 ± 0.039 (28 persons); and Lake Preston, 0.26 ± 0.058 (22 persons). The plasmas of 24 persons hospitalized because of a variety of chronic illnesses were found to have fluoride contents not in excess of the range of local normal plasma fluoride concentration, except possibly for 3 cases of demineralizing disease of the skeleton (hyperparathyroidism and multiple myeloma). The elevation of plasma fluoride in these cases was regarded as a result of the disease.

VI. Enamel and Dentin: Physical and Chemical Properties. March 20, Forenoon, Section C.

55. Microradiographic and Micro-Hardness Studies on Fluorosed Enamel.—*Ernest Newbrun, Medical Center, University of Alabama, Birmingham, and Finn Brudevold, Harvard School of Dental Medicine, Boston.* Previous research has been directed mainly toward chemical differences between fluorosed and normal enamel. The purpose of this investigation was to study certain physical properties of fluorosed enamel. Thirteen fluorosed permanent teeth collected from areas where the water supply contains 2.2 to 5.0 ppm F^- were studied microradiographically. The location and degree of radiolucency were classified and compared to the macroscopic appearance. With one exception, all the teeth studied showed macroscopic evidence of mottling in the form of opaque white, white-yellow, and brown areas. Most of these teeth showed areas of radiolucency which were always subsurface, the outer layer being well calcified. Where the drinking water contained less than 5.0 ppm of fluoride only the outer one third of the subsurface of the enamel was radiolucent. Teeth from Post, Texas (5.0 ppm F^- in drinking water) showed radiolucent areas extending through one half to three quarters of the enamel thickness. The hardness of 30 fluorosed permanent teeth was measured, using a Kentron microhardness tester with a 500 g load applied for 10 sec. A total of 344 readings was carried out on these teeth. A Dental Fluorosis Index (DFI) was devised on the basis of the macroscopic appearance of the mottling. The Knoop Hardness was found to be inversely proportional to the degree of dental fluorosis. The average Knoop Hardness of teeth from Post, Texas (5.0 ppm F^- in drinking water) was 305 ±24, which is significantly lower than values (367 ±35) obtained for teeth from Birmingham, Alabama, where the fluoride content of the drinking water is very low (0.00 to 0.03 ppm). The hardness of teeth from Doland, South Dakota (2.0 ppm F^- in drinking water) averaged 355 ±20, and of teeth from Alamosa, Colorado (1.5 ppm F^- in drinking water) averaged 396 ±18. These latter two values are not significantly different from the average hardness of Birmingham teeth. It was concluded that enamel formed in areas where the fluoride content of the drinking water is high is not harder than enamel formed in other areas. Extreme mottling of fluorosed teeth is associated with an actual lower hardness than normal enamel.

56. Some Chemical and Physical Properties of "Altered" Enamel.—*Marguerite F. Little and Eriberto J. Cueto, Eastman Dental Dispensary, Rochester.* This study was designed to determine whether stained, white, or brown spot enamel differs markedly from normal enamel in physical or chemical properties. Teeth (150) showing white spot, brown spot, or extensively

stained enamel were split through the center of the area. Half of each area was removed by grinding and pooled with other areas of the same type. The other half was sectioned, exposed to soft-x-ray and the radiolucent area carefully dissected, using airbrasive and fine instruments. Normal enamel from the same tooth was used as a control. Nitrogen and CO_2 analyses were done on each of the ground samples and on the pooled dissected areas of each type. Stained, white, and brown spot areas all exhibited a characteristic subsurface radiolucency to soft x-ray. The depth of the area into the enamel was greatest in brown spot lesions, less in white spot lesions, and least in stained enamel. Most of the stained enamel sections showed wide shallow lesions extending under much of the enamel surface. In all types of "altered" enamel, the nitrogen content was higher (0.2 to 0.3 per cent) than the controls (0.07 to 0.11 per cent), while the inorganic CO_2 (1.2 to 1.8 per cent) was lower than the controls (1.5 to 2.5 per cent). Refractive index measurements on the ground samples of altered enamel showed 400 to 500 particles per 1,000 below a refractive index of 1.58. The controls were all above 1.58 and only 10 particles per 1,000 were below a refractive index of 1.60. Preliminary studies with polarized light tended to confirm this observation.

57. AMINO SUGARS IN DENTIN AND ENAMEL.—*G. Nikiforuk, R. C. Burgess, and C. Maclaren, Division of Dental Research, University of Toronto, Toronto, Canada.* Hydrolysates of enamel organic fractions were analyzed for amino sugars by paper chromatography technics. The results were inconclusive due to the presence of large amounts of amino acids. The interference from amino acids was overcome by the use of ion exchange columns; glucosamine and galactosamine were quantitatively separated by this method. The procedure was as follows: enamel powder, purified by the Manly-Hodge procedure, was demineralized against ethylene diamine tetracetate (pH 7.1) then separated into soluble and insoluble organic fractions by centrifugation. These fractions (10 to 50 mg.) were hydrolyzed with Dowex 50 X.12 (500 mg.) and 1 ml. 0.05 N HCl in a sealed glass ampule at 105° C. for 24 hours. The resin was transferred quantitatively to the top of a Dowex 50 X.12 column (60 cm. by 1.2 sq. cm.) and the column eluted with 200 ml. 0.3 N HCl. The eluate was collected in 3 ml. fractions; 0.5 ml. aliquots were analyzed for hexosamine by a modified Elson-Morgan reaction. By this procedure, enamel organic matter was found to contain approximately equal quantities of glucosamine and galactosamine. The hexosamine compositions of soluble and insoluble organic fractions were qualitatively similar. Dentin was found to contain galactosamine and glucosamine in the soluble and insoluble organic fractions. The dominant amino sugar in dentin was galactosamine.

58. COMMON ION EFFECTS ON ENAMEL AND DENTIN SOLUBILITY AT VARIOUS HYDROGEN ION CONCENTRATIONS.—*Apostolos X. Apostolopoulos and Michael G. Buonocore, Eastman Dental Dispensary, Rochester.* Since no comparative studies of common ion effects on enamel and dentin solubility rates have been done, this matter was investigated. Solubility rates of 50 mg. samples of 200 to 270 mesh enamel and 68 mg. of 120 to 200 mesh dentin in 50 c.c. of 0.1 M acetate buffers at pH 3.5, 4.0, and 5.0 were compared at intervals of 10, 20, 30, 40, 50, and 60 minutes by continuous shaking in specially designed reaction-filtration flasks (Series I). Next, the effect on solubility rates of the periodic removal of calcium and phosphate ions from the decalcifying medium was studied (Series II). In this series, the buffer was filtered off at each 10-minute interval, the enamel particles washed, suction-dried, and re-exposed to fresh buffer for an additional 10 minutes. The combined washings and

original filtrates were analyzed for Ca and P. The solubility values in Series II, corresponding to the Series I time intervals, were the sum of the Ca and P dissolved in each 10-minute interval. In Series I, at pH 3.5, enamel and dentin showed about the same dissolving rates. At pH 4.0, enamel and dentin dissolution rates were the same during the first 20 minutes; thereafter dentin dissolved more rapidly than enamel. At pH 5.0 over the whole reaction period, the dentin solubility rate was approximately twice that of enamel. In Series II, however, the removal of common ions at pH 3.5 and 4.0 resulted in an interesting reversal of behavior; the dentin now dissolved at a considerably slower rate than enamel. At pH 5.0, the dentin solubility rate was now only slightly greater than enamel. These results suggest that the presence of common ions depresses the rate of enamel solubility considerably more than dentin.

59. The Carbohydrate Content of Caries-Susceptible Rat Molars.—*Al Wilcox, Pat O'Day, and M. I. Haley, College of Medical Evangelists, Loma Linda, and R. R. Steinman, CME School of Dentistry, Loma Linda, Calif.* Numerous investigators have demonstrated a direct relationship between sucrose and dental caries. Since a high sucrose diet will produce appreciably more caries susceptibilty than a Purina diet, it was of interest to compare the total carbohydrate content of the molars of rats on the two diets. After weaning at 21 days of age, 18 litters of albino rats were divided and half of each litter was placed on stock Purina ration and the other half on a 65 per cent sucrose cariogenic diet. At intervals of 1 week from 29 to 49 days of age, groups of littermates on both diets were sacrificed and the total carbohydrate content of their molars determined. The carbohydrate determinations were carried out on the thoroughly cleaned and vacuum-dried first and second molars of each animal by a modified colorometric anthrone technic and expressed in micrograms of carbohydrate per 100 mg. of teeth. At the 28-day age level, after the animals had been on the two diets for 1 week, the teeth of 44 Purina-fed rats had a mean carbohydrate content of 325 μg of carbohydrate per 100 mg. of teeth, whereas the teeth from 46 sucrose-fed rats had a mean score of 247 μg of carbohydrate per 100 mg. of teeth. The amount of carbohydrate in the teeth of the Purina-fed group was significantly greater ($p = 0.001$). Comparable studies were made on the two diet groups at 35, 42, and 49 days of age, but no significant differences were seen in the carbohydrate content of the teeth of the groups at the later age levels.

60. Effect of Age and Exposure to Oral Environment on the Permeability of Teeth.—*L. S. Fosdick, R. Q. Blackwell, and C. Wachtl, Northwestern University Dental School, Chicago.* Single rooted teeth were set up as a cell in such a manner that they could be used as semipermeable membranes. Distilled water was placed in contact with the pulp side or inner surface of the membrane. Solutions of ammonia or urea were placed in contact with the sound enamel surface or outer surface of the membrane. After definite time intervals, the solution from the inner surface was chemically analyzed for the test material and the rate of passage through the tooth determined. It was found that: (1) the rate of diffusion through the tooth was a direct function of the concentration of the diffusible species in contact with the enamel surface; (2) the permeability of the tooth was a function of age—the older the tooth, the less permeable it was, and (3) impacted old teeth, that had not been exposed to the oral fluids, were of the same order of permeability as freshly erupted teeth.

61. RATE OF PENETRATION OF HUMAN ENAMEL BY ACIDIC BUFFERS.—*Krishan K. Kapur, William H. Emerson, and Richard S. Manly, Tufts University School of Dental Medicine, Boston.* Studies were made on birefringence changes in tooth enamel sections subjected to decalcification on the external surface for periods up to 1 month. Measurements, in microns, of the distance of penetration toward the pulp were obtained by projection of Kodachrome transparencies taken at different time intervals. Ground sections of human teeth, 125 μ thick, were mounted between glass slides with epoxy resin. Small areas of the external enamel of each section were exposed and the section subjected to the action of buffers for periods up to 1 month. Lactate and acetate buffers were used at pH 4, 5, and 6. Each experiment involved a 4 by 4 study, four different molarities of acid and four different degrees of saturation with tricalcium phosphate for each molarity. Agitation of the buffer solution seemed to have a minor influence on rate of penetration. Under certain conditions, decalcification was observed to penetrate to the enamel dentin junction without producing any cavitation. In one series of tests, a constant rate of decalcification (expressed in microns per hour) was observed for periods up to 600 hours. Rates of penetration toward the pulp were greater with buffers having a lower pH, but the relative amounts of cavitation and decalcification were determined by saturation and molarity in a complex manner.

62. TIME LAPSE CINEMATOGRAPHY OF EDGE DECALCIFICATION OF TOOTH SECTIONS.—*William H. Emerson and Richard S. Manly, Tufts University School of Dental Medicine, Boston.* A method is presented for studying, by time lapse photography, the change in birefringence of tooth enamel while it is being decalcified by acidic buffers applied to the exposed exterior surface. Ground sections of human teeth, 125 μ in thickness, were mounted between glass slides with epoxy resin. A small area of the exterior enamel was exposed by removal of resin. The slide was mounted on the stage of a polarizing microscope and exposed to buffered lactate solution, pH 4 to 6. A movie camera was aligned with the microscope on an optical bench and the slide illuminated by transmitted light from a 100-watt lantern. A three tube electronic timer was especially constructed for the purpose of turning on the light and then exposing a single frame. This operation occurred at periods ranging from 30 seconds to 12 minutes and could be continuous for an indefinite period. The first trial of the equipment provided a 1-minute film showing gradual motion of the positively birefringent area as decalcification proceeded half way into the enamel layer.

63. THE PRIMARY CARIES LESION.—*A. F. Forziati, Research Division, National Bureau of Standards, Washington, D. C., W. H. Jennings, and F. L. Losee, Naval Medical Research Institute, Bethesda.* Several hundred teeth were serially sectioned and studied by means of polarized and fluorescence microscopy, and microradiography. In many instances, areas of enhanced fluorescence and altered birefringence were found in the enamel, although it was not possible to detect any loss of mineral content by microradiography or with a clinical explorer. These areas, then, might have been the primary or preclinical lesion of early caries. The results of the study suggest that this primary lesion was due to some organic agent, initiating from the accumulation of oral material on the enamel surface, which diffused into the enamel and produced the effects described. The form of the carious lesion was shown to be influenced by the microscopic structural features of the enamel and to

have existed in geometrical forms quite different from the "classical" truncated cone. The hypermineralization of the surface of the carious lesion was also substantiated.

64. Investigation of Pattern of Mineralization of Enamel.—*J. K. Avery and R. Visser, University of Michigan School of Dentistry, Ann Arbor.* The object of the present investigation was to gain more information concerning the pattern of enamel calcification. The study was undertaken on human and Rhesus monkey enamel using three means of analysis: (1) microhardness pattern of enamel mineralization, (2) microradiography, and (3) plastic substitution studies. A series of teeth was selected for study. Half of each tooth was tested for microhardness on a Tukon Microhardness Tester with a Knoop Diamond Indentor. The microhardness indentations were of sufficient number so that all areas of the enamel of the developing crowns were evaluated. The other half of each tooth was further sectioned. Several sections from each tooth were ground to less than 150 μ in thickness and microradiographs were taken. The remainder of these sections were used for the substitution studies. All organic tissue (ethylenediamine extraction) and water were removed from the sections and replaced with plastic. Decalcification of these sections then revealed areas of low and high organic and water content. According to these means of analysis, it was found that mineralization of the enamel matrix begins at the dentinoenamel junction and proceeds peripherally. Thus, it closely follows the incremental pattern of formation of the enamel matrix. Final calcification is completed after the matrix is completely deposited and follows this same pattern from the dentinoenamel junction peripherally. Calcification is thus completed in the cusps before the intracuspal or cervical areas, therefore, simulating the pattern of matrix formation.

65. Preliminary Observations on the Reversibility of Incipient Enamel Demineralization.—*Reidar F. Sognnaes, Harvard School of Dental Medicine, Boston.* The theoretical concept for redeposition of minerals in enamel was reappraised on the basis of more recent observations on radioisotope exchange reactions between enamel and saliva in primate teeth. These experiments suggest that an equivalent mechanism operates during the final submicroscopic crystal growth of the enamel of erupting teeth. Experimental application of acid to the enamel surface of teeth in vivo resulted in demineralization in depth beyond what could be detected by gross inspection or by surface replica technics. In the living animal, the demineralization had a definite preferential pattern along the Retzius lines where it penetrated to a considerable depth before grossly visible loss of surface contour. In the normal Rhesus monkey, these areas of demineralization appeared to be prone to redeposition of minerals, demonstrable within a week or 2. These areas, despite the regained high microdensity, appeared to contain an organic substance which has a greater dye-binding capacity than intact mature enamel. It is believed that enamel in which minerals are redeposited, following experimental demineralization, is not completely reconstituted to its original form, and that it would be inappropriate to label this mechanism as a "genuine healing process." There appeared to be clinical, theoretical, and experimental bases for the hypothesis that incipient biochemical destruction of the enamel, even though it extends along certain susceptible pathways below the external enamel surface, has potentialities for reversibility within certain limits yet to be fully explored in theory and exploited in practice.

66. DIFFUSION OF NIACIN THROUGH UNERUPTED HUMAN TEETH AND ITS EFFECT ON BACTERIAL PENETRATION INTO DENTIN.—*L. R. Brown, L. W. Wachtel, and M. G. Wheatcroft, U. S. Naval Dental School, Bethesda.* The purpose of this study was to determine whether an essential metabolite would diffuse through the dentin of whole teeth in sufficient quantities to support bacterial growth. Two plastic tubes were attached separately to each tooth in order to seal externally the crown portion of the tooth from its root portion. Dentin was exposed by drilling through the enamel by means of a No. 2 bur. Solutions containing 100 μg of niacin per milliliter were placed on the root sides of the teeth; niacin-free solutions were placed on the crown portions. Samples of the solutions in contact with the crowns were removed and niacin content was determined by microassay technic, using *Lactobacillus arabinosus* 17/5. Niacin was found to have diffused through the dentin of each tooth at a constant rate averaging 0.13 μg per day (0.07 to 0.25). Undrilled teeth used as controls averaged 0.04 μg per day (0.02 to 0.05). In order to determine if *L. arabinosus* would penetrate the dentin, a series of teeth was prepared in which the crowns were covered with a niacin-free medium, and control teeth were covered with a complete medium; all media in contact with the crowns were inoculated with the test organism. After periods up to 12 weeks the teeth were sectioned and stained in order to study dentinal changes. Where a medium containing niacin was used on the crowns of the teeth, no penetration of organisms was detected. Where the niacin-free medium was used, and the supply of niacin depended upon diffusion from within the tooth, organisms were found to have penetrated into the dentin, and dentinal changes similar to those noted in caries were observed.

VII. HATTON AWARD COMPETITION I. MARCH 20, AFTERNOON, SECTION A.

67. ERUPTION OF ROOTLESS TEETH.—*Joseph M. Gowgiel, Zoller Dental Clinic and Department of Anatomy, The University of Chicago.* In a series of monkeys that received external fractionated irradiation, totaling 4,500 r to 7,500 r, to the maxilla and mandible, it was observed that further root and crown formation of developing teeth was completely inhibited. The animals varied in ages from 14 to 28 months and therefore all permanent teeth were involved. The resultant size and shape of the permanent teeth were dependent upon their state of development at the time of irradiation. The irradiation did not affect calcification of the crown matrix. Eruption of the partially formed roots and crowns was observed by means of lateral jaw x-rays taken at regular intervals. These teeth appeared to erupt at a somewhat retarded rate into full functional occlusion, and the deciduous roots resorbed normally. A few partially formed crowns were supported only by the gingiva. Layers of cortical bone were found under all erupting teeth. Some of the partially formed teeth did not erupt. Histologic examination revealed two types of dentin—normal, and a "trabecular" type. The odontoblasts and Hertwig's epithelial root sheath ended abruptly at the junction of the normal and abnormal dentin. The pulp was normal around the odontoblasts and more fibrous in the inferior part. The crowns did not have periodontal ligaments. They were supported by fibers of the dental sac which were attached to the cementum.

68. THE PSYCHOLOGIC REACTION OF CHILDREN TO LOCAL ANESTHETIC PROCEDURES IN THE ORAL CAVITY.—*A. L. Ingino, The University of Kansas City School of Dentistry, and The Children's Mercy Hospital, Kansas City, Mo.* The

emotional response elicited following the use of "needles" is an experience familiar to every practitioner in dentistry and medicine. The emotional response evoked by the use of hypodermic needles and local oral injections is more pronounced in the practice of pediatrics and pedodontics. A study was devised to assess the subjective response in children from the viewpoint of oral injections of a local anesthetic. The same type of anesthetic agent was used in a Carpule syringe with a 27 gauge needle, and without presedation or topical anesthesia. The patient was made fully aware of the presence of the syringe and the intentions of the operator. One hundred eighty-four randomly selected patients, ranging in age from 5 to 15 years, constituted the study group. It was revealed that apprehension and fear declined as the age of the child increased, except in the 7-year-old, where the frequency rose, Pre-injection fear reached a peak in the 6-year-old, as did fighting defense reaction during injection. All of these reactions were greatly diminished in the older child. Post-injection resentment was greatest again in the 6-year-old, and was not a pronounced emotional response in any other age group. The factors that may have been involved in the formation of these reactions will also be presented.

69. The Role of Ascorbic Acid in the Maintenance of Blood Vessel Walls Studied in Polyvinyl Sponge Implants: Preliminary Report.—*Joseph M. Stolman, Boston University School of Medicine, Boston.* Small blood vessels were studied in regenerating and pre-existing tissues in normal and ascorbic acid-depleted guinea pigs, utilizing the technic of implanted polyvinyl sponges. Morphologic differences between the ascorbic acid-treated and ascorbic acid-depleted specimens were found in the blood vessel walls in the endothelial cells, smooth muscle cells, and supporting tissue fibers. The most significant finding in the preliminary study was that the connective tissue fibers in vascular and perivascular areas in the animals on ascorbic acid-free diets were disorganized. This observation was most pronounced in tissues that were undergoing or had undergone healing. There was some indication of similar disorganization, but to a lesser degree, in pre-existing tissues, which would tend to correspond with a slower rate of turnover in pre-existing tissue than in healing tissue. Although morphologic changes in the vascular walls and perivascular areas were shown in this preliminary experiment, it was not possible to determine the exact mechanism of vascular fragility. Further studies into this problem with more specific staining methods, therefore, have been initiated.

70. Failure of Calculus Formation in Rats on a Diet Containing Human Saliva.*—*Edward F. Miller,** Ohio State University, Columbus.* A relationship between human saliva and calculus formation has been established in in vitro studies. Albino rats, which are not susceptible to calculus formation, were used to test further this hypothesis. Two groups of 6-month-old inbred albino rats were utilized.† One group was fed a diet of commercial pellets softened with water to a soup-like consistency. The second group received a diet of similar pellets softened to the same consistency with human saliva secured from calculus formers. This soft diet, with the microorganisms and other elements of human saliva in the alkaline environment

*The contents of this article are the personal views of the author and are not to be construed as statement of official Air Force policy nor as Air Force indorsement of any commercial product described.

**Edward F. Miller, Captain, USAF (DC), assigned as graduate student in periodontology, Ohio State University.

†Experiments were conducted according to the "Rules Regarding Animal Care," as established by the American Medical Association.

of the rat's mouth should have presented the elements which favor calculus formation. Clinical examinations were made during the 90-day experimental period. At necropsy, the maxilla and mandible were studied under a dissecting microscope. Histologic examination of the teeth and surrounding tissues followed. Varying amounts of plaque were found in half of the sections of both experimental and control groups. No evidence of calculus, or caries, was found.

71. PRETREATMENT OF TEETH WITH SODIUM FLUORIDE PRIOR TO STANNOUS FLUORIDE APPLICATION.—*Hashim Abdlul-Ghaffar, Indiana University School of Dentistry, Indianapolis.* Laboratory studies using both powdered human enamel and whole teeth have suggested that the pretreatment with sodium fluoride immediately preceding the application of stannous fluoride increased the enamel's resistance to acid decalcification over that observed for stannous fluoride alone. Since the final criterion of any similarly developed anticariogenic agent is its effectiveness in human beings, a clinical test was conducted using 602 6- to 15-year-old children in Spencer, Indiana. The children received a dental examination and were divided into three groups on the basis of their past dental caries history. Group 1 received a single application of a 4 per cent aqueous sodium fluoride solution followed by a single application of an 8 per cent SnF_2 solution the following day. Group 2 received 1 application of an 8 per cent SnF_2 solution. Group 2 received 1 application of an 8 per cent SnF_2 solution on each of 2 successive days. Group 3 served as controls and received no treatment. All three groups received a thorough dental prophylaxis, and the first fluoride application was given immediately thereafter by methods which have been previously described in detail. After 1 year, the children were re-examined by the same dentist. The data showed that both types of fluoride treatment resulted in significant reductions in the incidence of dental caries, but the superiority of the pretreatment with sodium fluoride which was observed in vitro was not substantiated when the results were analyzed by either the DMFT or DMFS indices. Some reasons why human tests fail to substantiate animal tests and the need for accurate in vitro screening tests for human clinical tests are discussed.

72. EVALUATION OF ANTIAPPREHENSIVE DRUGS IN ADOLESCENT DENTAL PATIENTS.—*DeWayne Hunter, The University of Kansas City School of Dentistry and The Children's Mercy Hospital, Kansas City, Mo.* A double blind study was instituted on 105 normotensive adolescent dental patients. The patients ranged in age from 10 to 14 years. On the initial examination, the usual introductory dental procedures were performed and each patient was evaluated relative to apprehension, and given a coded package containing four 400 mg. tablets. The tablets consisted of ectylurea, meprobramate, or a placebo. Instructions were given to the patient to take the tablets at 6-hour intervals and to return to the clinic within 2 hours following the taking of the last tablet. The patients were then examined with the aid of a questionnaire for both subjective and objective responses, preceding and during an operative appointment. The confidence limits of these two drugs were compared to the placebo and found to be almost equal. A chi square test was performed evaluating each drug compared to the placebo. There was no significant difference in the effectiveness of any of the prescriptions in allaying anxiety in this group of patients.

73. AGE CHANGES IN HUMAN TEETH.—*John Nalbandian, Harvard School of Dental Medicine, Boston.* In a study on age determination of human teeth,

extending the work of Gustafson with comparable results, it became obvious that, of all the age changes, the sclerosis of root dentin was least affected by pathology. The changed appearance of the root dentin, its transparency in transmitted light, is generally held to be due to obliteration of dentinal tubules by intratubular calcification; but there has been no direct demonstration of such sclerosis by observations on calcification density. Electronmicrographs of *decalcified* dentin suggested what is now interpreted to be a highly acid soluble zone in the peritubular region of the tubules, because microradiographs of *undecalcified* dentin revealed distinct evidence of sclerosis. Small sections of dentin, from a region approximately half way between pulp and cementum, were selected for the microradiographic part of the study from three sources, as follows: (1) apical dentin from young teeth of individuals, aged 10 to 15 years; (2) highly transparent apical dentin from the teeth of adults and very old individuals; and (3) dentin at the junction of transparent and opaque zones. These specimens were ground to about a thickness of 50 μ, microradiographed on Kodak spectroscopic film 649 GH, at an average of 4 kv., 4 Ma, and 8 minutes. Observations on the microdensity showed a high degree of tubular obliteration by dense mineral deposition in the very transparent zones of old tooth roots, fewer obliterated or only partially obliterated tubules in the opaque zones, and a general absence of obliteration and dense peritubular rings in young apical dentin.

74. Formation of Periodontal Tissues Around Subcutaneously Transplanted Hamster Molars.—*Richard L. Hoffman, University of Illinois, Chicago.* To determine whether the stimulus for periodontium formation is located within or outside the developing enamel organ and dental papilla, hamster molar tooth germs were transplanted homologously into the subcutaneous tissue of hosts 30 days of age. Transplants consisting of the enamel organ and dental papilla, but not the dental sac, were transferred from 13-, 14-, and 15-day in utero newborns, and 5-day-old donors in which no periodontium had yet been formed. Older molars with developed roots and formed periodontium were also transplanted, after removing any bone between the roots, to determine how long the tooth might have the capacity to stimulate peridontium regeneration. Transplants totaled 112; 59 were recovered after 28 days of growth and development in the host. Of recovered transplants, 72 per cent showed formation of periodontal ligament and alveolar bone in the tissues organized around their roots. The stimulus for periodontium formation, therefore, seems to reside in the developing tooth and may be associated with the enamel epithelium and proliferating epithelium of Hertwig's sheath. Of special interest was the observation that even though the transplanted teeth had developed entirely within the loose connective tissues under the skin, the fiber orientation of their periodontal ligaments was found to be similar to normal teeth which had developed and functioned in situ. The amount of mechanical stress needed to orient the fibers of the periodontal ligament may be much less than that normally experienced in mastication.

75. Carbohydrate Retention in Interproximal Spaces of Posterior Teeth.—*Stanley Keller and Aage Jorgensen, University of Alabama School of Dentistry, Birmingham.* Attempts were made to determine the difference in retention of carbohydrate candies in the interproximal spaces of posterior teeth. The test subjects cleaned their teeth by brushing with baking soda and salt and rinsing with water. Half of the teeth were cleaned interproximally with dental floss to determine the efficiency of flossing. Subsequently,

the test subjects chewed different kinds of commercial and homemade candy. At intervals of 5 minutes, samples were taken from the interproximal space by means of a wire loop. These were tested for carbohydrate using the triphenyltetrazolium chloride test. Carbohydrates were detected consistently for 15 to 30 minutes longer on the nonflossed side. In an effort to study the localization of minute food particles within the interproximal space, small removable dental enamel slots were fitted to the mesial surface of full molar crowns. After a negative interproximal sample with the wire loop technic, the enamel slabs were removed and tested for carbohydrate. Supplementary observations were made on a test subject, who wore a partial denture with removable teeth. At specific intervals after confection ingestion, these teeth were removed and tested for the retention of carbohydrate. It was observed that very sticky candies were retained for periods up to 2 hours, while nonsticky candies were eliminated in 20 to 60 minutes. It was found that the use of dental floss reduced the retention time as much as 30 minutes.

VIII. Caries: Animal Studies. March 20, Afternoon, Section B.

76. Caries Initiation and Promotion Factors in the Rat.—*Willard G. Clements, Edward C. Hilger, Jr., John F. Stasik, Sara D. Goodlin, and Gerald J. Cox.—School of Dentistry, University of Pittsburgh, Pittsburgh.* Active carious lesions in first and second molar teeth of rats were found, in a previous study, to contribute to the initiation of carious lesions in third molar teeth, but maturation of the latter teeth had been effected on the nutritionally poor Hoppert-Webber-Canniff coarse rice ration. Six groups of rats were maintained for 8 weeks on 15 combinations of fine and coarse rice rations and fine and coarse ⅔ sucrose rations. The above previous conclusion was verified. However, third molar teeth, maturated on the H-W-C ration, were more susceptible to the initiation of the carious process if carious lesions were present than teeth maturated on the nutritionally better ⅔ sucrose ration.

77. Nutrition, Tooth Morphology, and Dental Caries in Rats.—*K. J. Paynter, Faculty of Dentistry, University of Toronto, and R. M. Grainger, Division of Medical Statistics, Ontario Department of Health.* The relation between nutritionally effected variations in tooth morphology in rats and their subsequent caries experience was investigated. The information was gathered from litters from (1) a control group of mothers fed laboratory chow during pregnancy and lactation, and (2) an experimental group of mothers fed a diet containing a high PO_4/Ca ratio during the same period. At 21 days of age, the young rats were anesthetized and the upper right first molar tooth was extracted from each animal. The extracted teeth were fixed in formalin, and stored in alcohol for later processing. The young animals were kept on a synthetic diet containing 60 per cent refined sucrose as carbohydrate for a period of 180 days, then sacrificed. The number and extent of cavities in each animal were recorded. Photographs were made of the extracted teeth under conditions of standard enlargement, and measurements of the teeth in the photographs were compared with the same animal's caries score. From a preliminary graphic analysis, it appeared that in the control group the number of cavities was directly correlated to tooth size, particularly in terms of the mesiodistal diameter of the crown at the cervix. The teeth with more cavities tended to be less bell shaped, and the ratio of their mesiodistal diameter to their buccolingual diameter tended to be higher.

Animals of the high phosphate group had smaller than average teeth which were considerably more caries-susceptible than the controls, both in number and extent of cavities.

78. Further Observation Regarding the Inoculation and Transmission of Experimental Animal Caries.—*Paul H. Keyes, National Institute of Dental Research, U. S. Department of Health, Education and Welfare, Bethesda.* To investigate the possibility of inducing caries by means of the inoculation of fecal material from caries-active animals, 48 "non-susceptible" animals were divided into three groups which were equalized in regard to the number of animals per cage, the caries-test diet (Abstract No. 177, I.A.D.R. Thirty-Fifth General Meeting, 1957), and weekly vegetable supplements. Sixteen control animals (Group A) were caged in groups of 3 or 4 and fed the test diet and distilled water ad libitum. An equal number of animals (Group B) were similarly treated but were mixed among other animals in the colony which were caries active. The remaining animals (Group C) received an inoculation of fecal material by means of either contaminated drinking water for 20 days or an intra-oral smear for 5 to 7 days. At the end of the experimental period, slight caries activity was found in the control group; only 9 animals were affected, with scores of 1, 3, 4, 5, 6, 8, 10, 11, and 30. All animals in Groups B and C developed active caries. Scores ranged between 30 and 150, with 27 animals with scores of 45 or more. In rats, 6 pregnant Osborne-Mendel females were equally divided. Three were maintained on the usual stock diet during the last few days of pregnancy and during the 25-day suckling period. The other 3 females and litters were fed the same stock formula into which were added 100 mg. of penicillin (Compenamine) per kilogram of ration. Upon commencement of the caries-test diet, 11 weanlings from the control litters and 17 from the antibiotic-fed litters were caged separately in groups of 3 or 4. In addition, 8 animals from the antibiotic litters were combined in the cages with controls. At the end of the 50-day experimental period, the average caries scores for the control animals, the antibiotic-fed group, and the combined group, respectively, were: smooth surfaces 23.4, 1.2, and 7.8; proximal 5.6, 0.5, and 6.5; major sulcal 27.4, 15.4, and 30.3. The average numbers of minor sulcal lesions were 6.6, 0.0, and 6.5. These findings suggest that experimental dental caries can be induced by inoculation, and it can be transmitted between members of the same strain but with uncertainty between different strains.

79. A Comparison of Caries Susceptibility in Hamsters, Rats, and Cotton Rats.—*Philip J. Holloway, Harvard School of Dental Medicine, Boston.* The relative caries susceptibilities of 3 species of experimental rodents were evaluated. Hamsters, rats, and cotton rats, housed and bred in the same building under similar regimens, were fed cariogenic diets for comparable periods, and their caries experiences compared. One hundred and ninety-five hamsters were fed 1 of 6 high-sugar diets at various stages in their development, and under different environmental conditions for 100 to 300 days. Only 22 cavities were seen in the teeth of these hamsters. Forty-eight white rats were fed 1 of 2 high-sucrose, synthetic diets, beginning at different times during their development. One hundred and forty gross lesions were present in the molar teeth after 200 days on experiment; 126 occurred in the mandibular first and second molars. Twenty cotton rats were fed a high-sucrose, synthetic diet from weaning for 205 to 250 days. One hundred and twenty-seven lesions were seen which were distributed evenly between the maxillary and mandibular molars. Within the particular strains of the 3 species tested, the cotton rat was the most susceptible and the hamster the most resistant.

In comparison with reports from other laboratories, these representatives of all 3 species showed a relatively high resistance. No generalized statement, however, can be made on the over-all caries resistance of these 3 species; indeed, the caries experience encountered in any study will be a composite of the genetic constitution of the strain used in relation to the environment in which it developed and was maintained.

80. ADDITION OF SALIVA FROM CARIES-IMMUNE AND CARIES-SUSCEPTIBLE HUMAN BEINGS TO DESALIVATED ALBINO RATS.—*Harry Blechman, Om P. Gupta, and Henry A. Bartels, Murry and Leonie Guggenheim Foundation, Institute of Dental Research, New York University, New York.* One hundred and twenty weanling Sprague Dawley rats were divided into six experimental groups, each group consisting of an equal number of males and females. Three groups of these animals were sialoadenectomized, one group was sham operated and the remaining two served as the intact control groups. Stimulated whole saliva was collected on a daily basis from two groups of human volunteers, caries-immune and caries-susceptible. An aliquot of each of the pooled salivas was diluted with an equivalent amount of tap water daily and fed to the appropriate experimental group. All of the groups, but one of intact controls, were maintained on a modified Stewart, Hoppert, and Hunt powdered, purified ration consisting of 67 per cent confectioner's sugar, 20 per cent whole powdered milk, 3 per cent desiccated liver, 5 per cent alfalfa, 2 per cent brewer's yeast, and 3 per cent H.M.W. salt mixture. All animals were housed in individual metal cages and received their respective diets and drinking fluid ad libitum for a period of 12 weeks. At the end of the experimental period, the animals were sacrificed by ether inhalation and the extent of dental caries scored. The desalivated animals maintained on a cariogenic diet showed a statistically significant increase in the incidence of dental caries as compared to intact and sham operated controls. The addition of pooled saliva from caries-susceptible individuals to the drinking water fed desalivated rats resulted in a significant increase in tooth destruction. Pooled saliva from caries-immune individuals resulted in a decrease in the average extent of carious lesions. This decrease was not statistically significant at the 5 per cent level. Real differences were obtained when the caries-susceptible and caries-immune group were compared. It was concluded that saliva contains a factor or factors which modifies the extension of dental caries in the desalivated albino rat.

81. CARIES SUSCEPTIBILITY INDUCED IN PREWEANLING RATS BY SIMULATED RAT MILK FORMULA.—*Pat O'Day and M. I. Haley, College of Medical Evangelists, Loma Linda, Calif.* Since it has been established by numerous investigators that there is a direct relationship between refined carbohydrates and caries, it was of interest to investigate the systemic caries susceptibility produced in preweanling rats by four carbohydrates incorporated into a simulated rat milk formula. Four groups of preweaning rat littermates were fed, from 14 to 21 days of age, a simulated rat formula. They were housed in specially designed heated cages. Group 1 had 5.35 per cent lactose added to the basal simulated formula, Group 2 had 5.35 per cent fructose, Group 3 had 5.35 per cent Dextri-maltose, and Group 4 had 5.35 per cent Karo added. All groups were weaned at 21 days of age and placed on a 64 per cent sucrose cariogenic diet. All animals were sacrificed at the end of 14 weeks and their molars scored for caries damage. No significant difference was observed between the mean weaning weights of the groups. Group 1, which had lactose added to the formula, had the lowest caries incidence (mean

caries score of 9.6). Group 2, which received fructose, had a slightly higher score (14.7) but was not statistically different from Group 1. Group 3, which received Dextri-maltose, had a mean caries score of 26.4, which represented a significant increase ($p > 99$ per cent) over the lactose susceptibility. Group 4, which had Karo added to its simulated formula, had the highest mean caries score (33.2), which represented a very significant increase ($p > 99.9$ per cent) in caries susceptibility in comparison to the addition of an equal amount of lactose.

82. Effect of Different Dietary Levels of Metaphosphoric Acid on Hamster Caries.—*A. E. Nizel and R. S. Harris, Dept. of Food Technology, Massachusetts Institute of Technology, Cambridge.* A caries-producing diet containing 0.37 per cent total phosphorus was supplemented with glacial metaphosphoric acid to raise the phosphorus content to 0.56 per cent, 0.74 per cent, and 1.47 per cent. These four diets (containing 1 P, 1.5 P, 2 P, and 4 P) were fed to hamsters beginning at 8 days of age for more than 75 days. Caries was scored in vivo, periodically. Highly significant protection against caries was observed in all supplemented groups, and the degree of protection was correlated with the level of phosphorus in the diet. A significant decrease in caries was observed in the diet containing 1.5 P, essentially complete protection with 2 P, and no caries with 4 P. The body weight gains of all groups were the same and no gross differences were observed. These results confirm a previous study with a different caries-producing diet in which the phosphorus content (0.53 per cent) was doubled by supplementation with HPO_3.

83. The Relative Cariogenicity of Diets of Different pH Levels.—*Winfrey Wynn, John Haldi, K. D. Bentley, and M. L. Law, Department of Physiology, Emory University, Atlanta.* Three high-sucrose diets were fed to 30 groups of desalivated littermate rats, from weaning, for a period of 70 days. Diet 1 was the Harvard-100 diet, Diet 2, the Harvard H-100 to which was added 3 per cent Na_2CO_3, and Diet 3, the Harvard-100 to which was added 1.2 per cent lactic acid. The pH of each diet as determined with a glass electrode immersed in 50 grams of the diet suspended in 50 ml. distilled water was for Diet 1, 6.0; Diet 2, 8.9; Diet 3, 4.7. The average caries scores of the animals fed these diets were 54, 31, and 68, respectively, on Diets 1, 2, and 3. The antimony electrode was used to determine the pH on the surfaces of the teeth in the early part of the experiment before the animals had developed caries. The average pH readings on the tooth surfaces of the animals fed Diets 1, 2, and 3 were 6.7, 7.3, and 6.3, respectively. These data indicate that making this high-sucrose diet alkaline reduces its relative cariogenicity, while making the diet more acid does not have a significant effect on its cariogenicity.

84. Effect of a Fat-Free Diet on Bones, Periodontium, and the Incidence and Severity of Caries in the Immature Rat.—*L. A. Bavetta, R. B. Alfin-Slater, S. Bernick, and B. H. Ershoff, University Southern California, Los Angeles.* Three groups of rats were employed in the present experiment. Group I was fed a purified fat-free basal ration consisting of sucrose, 71 per cent; hot-alcohol extracted casein, 24 per cent; Hubbel, Mendel, and Wakeman salt mixture, 5 per cent; and the known vitamins. Group II was fed the basal ration plus 10 per cent cottonseed oil, and Group III was fed the basal ration plus 10 per cent of a completely hydrogenated cottonseed oil. The fats were added in place of an equal amount of sucrose. Male and female rats of the Holtzman strain were selected at 23 to 25 days of age and were fed

the above diets ad libitum for 90 days. The incidence and severity of caries were significantly greater in rats fed the fat-free diet (Group I) than in animals fed the fat-supplemented rations (Groups II and III). No significant difference was observed between animals fed cottonseed oil and the completely hydrogenated cottonseed oil with respect to caries incidence and severity, suggesting that the protective effect of dietary fat was not due to its content of unsaturated fatty acids. In addition to the higher incidence and severity of caries, rats fed the fat-free diet exhibited marked degenerative changes in the salivary glands. Degenerative changes as a result of the deficiency of dietary fat were also apparent in histologic sections of the long bones and periodontium, as evidenced by the existence of extensive osteoporosis as well as an increase of fatty tissue in the marrow space.

85. CARIES INCIDENCE IN CORTISONE-TREATED RATS.—*F. C. Besic, S. Zimmerman, W. Normore, L. Frazier, V. Carbonell, D. Mitziga, and F. J. Orland, Zoller Dental Clinic, The University of Chicago, Chicago.* This pilot study was designed as a basis for future determination of the effects of cortisone and other hormones upon caries activity in rats. Forty young male and female Lobund strain white rats were divided into 4 different dietary groups and sustained for 85 to 87 days with the following results.

	GROUP I	GROUP II	GROUP III	GROUP IV
	(CONTROL) L-128 DIET	L-128 DIET + 5.0% SUCROSE SOL.	L-128 DIET + CORTISONE ACETATE	L-128 DIET + 5.0% SUCROSE + CORTISONE ACETATE
Total number of lesions	30	42	41 (37% increase)	33
Total score	59	140	86 (44% increase)	124

Caries results were obtained from 36 animals (9 in each group). Animals on cortisone acetate diets received 0.5 mg. per 15 grams of diet per day for 2 weeks, 1.5 grams for the next 3 weeks, and 2.5 grams for the remaining 7 weeks. No conclusions can be drawn from such a small experimental group, however, the 40 per cent (average) increase in caries score and number suggests justification for further investigation, perhaps with some modifications in procedures.

86. HISTOCHEMICAL ANALYSIS OF LESIONS IN INCIPIENT DENTAL CARIES.—*Ralph R. Steinman, CME School of Dentistry, and Gordon Hewes, CME School of Medicine, Loma Linda, Calif.* Six hundred caries-susceptible albino rats were weaned and divided into 2 groups at 21 days of age. One group was placed on a typical high-sucrose cariogenic diet, the other on Purina laboratory chow. The animals were sacrificed serially at 21 to 50 days of age. The jaws were frozen immediately and sectioned, while frozen, with a microtome in a Deepfreeze. One half of each tooth structure was shaved away, and the blocks of ice with one half of the jaw sections were mounted upside down and shaved until only a thin section 20 to 30 μ in thickness remained. The following histochemical technics were applied to these sections: the ninhydrin-Schiff method of Yasuma and Ichikowa for reactive proteins, the Hotchkiss periodic acid for polysaccharides, the Macallum method for sulphur, the method of Eranko for reducing agents (vitamin C), the method of Semenoff for succinic dehydrogenase, the method of Villamil and Mancini for cytrochrome oxidase, and the method of Quincke for iron. By 28 days of age, an

increase in reactive protein, carbohydrate, loss of mineral, cessation of oxidation reduction, absence of cytrochrome oxidase and an increase of iron in a considerable depth of the dentin beneath the enamel were observed in the depth of many of the major grooves of the animals on the cariogenic diet.

IX. Calcified Tissues: Histology, Chemistry, and Physical Properties. March 20, Section C.

87. The Formation of Mandibular and Maxillary Exostoses in Rats.—*Robert Van Reen and Harvey W. Lyon, Dental Division, Naval Medical Research Institute, Bethesda.* Molybdenum has recently been shown to be of importance in the nutrition of animals. This is probably a reflection of the findings that molybdenum is an integral part of several enzyme systems. At certain dietary levels, however, the mineral produces a toxicosis. This report deals with one of the unreported characteristics of sodium molybdate toxicity in rats, namely, the formation of mandibular and maxillary exostoses. It was observed that many of the NMRI-D strain of rats developed exostoses when fed molybdenum toxic diets, whereas none of the control animals developed the syndrome. The maxillary exostoses were found on the superior surface of the anterior portion of the zygomatic arch and were not as severe as the mandibular growths. The most pronounced exostoses occurred on the anterior aspect of the masseteric ridge of the mandible with smaller growths on the posterior portion of the ridge and also on the distal portion of the condyloid and coronoid processes. Several manifestations of molybdenum toxicity in rats have been shown to be mitigated by the inclusion of sodium sulfate in the diet, and it has been suggested that a specific molybdate-sulfate relationship exists. In the present study, it was found that one half of the rats receiving 0.4 mM. molybdate per 100 grams of diet developed mandibular exostoses, whereas rats receiving 2.0 mM. sodium sulfate in addition to the molybdate showed fewer growths.

88. Histochemical Observations on the Osteoclasts in the *ia* Rat.—*Anna Morse, Harvard School of Dental Medicine, Boston.* Numerous osteoclasts were found in the bones of the *ia* strain of rats in spite of the fact that these animals are characterized by a genetically transmitted failure in bone resorption. This cell population was distinguishable from that in the normal bone by the larger proportion of huge multinucleated cells and by the presence of many round osteoclasts lying free between the bony trabeculae. Various histochemical tests were employed on appropriately fixed tissues to determine whether the osteoclast of the *ia* rat reacted in the same manner as the normal osteoclast. The normal osteoclast contained materials which were pyroninophilic, variably basophilic, periodic acid-Schiff positive, and metachromatic. Most osteoclasts apposed to bone, and presumably functioning in bone resorption, contained an oxyphilic and pyroninophobic zone of variable width adjacent to the bone. The absence of the zone was a feature of the majority of the osteoclasts in the *ia* rat. A correlation between the reactions of this zone and osteoclastic function is being sought.

89. The Relative Effects of Vitamin C Deficiency Versus Starvation on Mandibular Condyle Osteoclast Numbers in Pair-Fed Guinea Pigs.—*William R. Johnson, Hugh I. Myers, and Ben W. Warner, University of Kansas City, School of Dentistry, Kansas City, Mo.* Seven pairs of animals, 48 days old, were used. The first member of each pair was given 25 grams of Nutritional Biochemicals Corporation's ascorbic acid-deficient diet and green vegetables for 4 days. They were then given the scorbutic diet without green

vegetables. The residual amount of scorbutic diet was determined 24 hours later. The second member of each pair was given the amount of scorbutic diet consumed by the first member the preceding day. This diet was fortified with green vegetables for the first 4 days, and 6 mg. ascorbic acid during the entire feeding period. This daily routine was continued until the animals were 72 days of age. At this time the animals were sacrificed and the mandibular condyles removed and histologic sections prepared, mounting every tenth section. The average number of osteoclasts counted for the 7 animals, given the scorbutic diet only, was 685, whereas the number for the 7 that were given the scorbutic diet, plus ascorbic acid, was 677. Previous studies have shown that the normal number of osteoclasts at 10 weeks of age was approximately 900. From this data, it would seem that the scorbutic diet definitely retarded the growth of the condyle and hence the modeling resorption engaged in by the osteoclasts was reduced, and further, that the ascorbic acid at the level administered had no influence in preventing this effect.

90. MICRORADIOGRAPHY OF ORGANIC BONE CONSTITUENTS IN NORMAL GROWTH.—*Richard C. Greulich, University of California Medical Center, Los Angeles.* As a part of a continuing histophysical investigation of the role of the organic matrix in the mineralization process, high-resolution ultrasoft microradiography was utilized to determine the distribution of water-free organic constituents in specimens of demineralized bone. Samples of normal bone from fetal, postnatal, and adult rats, together with comparable samples from mouse, monkey, and man were demineralized in disodium EDTA (pH 7.4) after fixation in neutral formalin. Paraffin sections were cut at 6 μ for histologic and microradiographic study. In order to compare the distribution of mineral mass with that of the organic matter, certain bones were not decalcified, but instead were embedded in methacrylate for preparation of ground sections. Ultrasoft microradiograms were prepared at 1.5 to 2.0 kv., utilizing a copper target (effective polychromatic wave length range, 6 to 20 A.U.), within an evacuated x-ray tube. Following exposure, many of the tissue samples were preserved for histologic comparison with their corresponding microradiogram. The undecalcified plastic sections were exposed to nickel-filtered copper radiation (effective wave length, 1.54 A.U.). The following results appeared to be of particular interest and will be dealt with in detail: (1) organic mass distribution in fully-formed bone matrix was remarkably uniform, regardless of its state of mineralization; (2) "physiological osteoid" surrounding bone matrix contained less organic mass than did the matrix proper; (3) primary "osteoid" laid down on cartilage in the metaphysis contained as much or more organic mass than fully-formed bone matrix; and (4) the content of organic mass in bone matrix immediately adjacent to active osteoclasts was greater than that of the surrounding bone matrix.

91. CHANGES IN SOLUBLE CITRATE OF PIGEON BONE DURING EGG-LAYING.—*Milton B. Engel, David Lehman, and Daniel M. Laskin, University of Illinois, College of Dentistry, Chicago.* During egg-laying there are alternating periods of bone apposition and resorption in the medullary cavities of the tibia and femur, which are accompanied by a rise in the citrate level of the blood. We considered that these simultaneous changes are primarily related to the citrate metabolism of the cells of bone. We, therefore, studied soluble citrate in medullary bone during various states of activity. Thirty pigeons were sacrificed during one of three stages: rest, apposition, and resorption. Cellular material was aspirated from the medullary bone of the tibia and femur using a fine-bore glass tube covered with a 100-mesh wire screen. This excluded the

large quantity of insoluble citrate in the extracellular part of bone. The aspirated material was homogenized in distilled water and then lyophilized. Citrate determinations were done on 50 mg. aliquots using L'Heureux and Roth's modification of the method of Natelson, Pincus, and Natavoy. In the resting stage, the citrate concentration was 167 μg per gram of dry aspirated substance. This level increased five times (846 μg per gram) during apposition and approximately eight times (1,361 μg per gram) in the resorptive stage. The foregoing data, and other experimental results, taken with the fact that citrate forms both soluble and insoluble complexes with calcium, led to the following hypothesis: During bone formation citrate arising from cellular metabolism can be deposited in the solid phase. When the concentration increases further, calcium is removed from bone salt and from its protein-bound complex to form soluble calcium citrate, and these events contribute to disintegration of the organized structure of bone.

92. Conversion of Pyruvate-2-C^{14} to Citrate in Bone Slices.—*Eugene C. Lekan, Daniel M. Laskin, and Milton B. Engel, University of Illinois, College of Dentistry, Chicago.* In studies on the citrate metabolism of bone, it has not been possible to distinguish the relatively inert extracellular citrate from the more labile intracellular part. This has hampered the understanding of relations between citric acid and bone resorption. To deal with this problem, the conversion of C^{14} labeled sodium pyruvate to citrate was studied in metaphyseal bone slices from normal rabbits and from a group in which bone resorption was produced by the injection of parathyroid extract (1,000 units over 48 hr.). Approximately 1,500 mg. of bone from the tibia and femur was incubated for 4 hours in a Krebs-Ringer bicarbonate buffer, pH 7.4, with 100 lambda of radioactive pyruvate-2-C^{14} at 37° C. The reaction was stopped by adding perchloric acid and the radioactive CO_2 was absorbed with KOH. The tissue was then homogenized and washed several times with additional perchloric acid. The DNA content of the residue was determined. The combined supernatants were neutralized with KOH and passed through a Dowex 1 (formate form) anion exchange column. Citric acid was eluted with formic acid according to the procedure of Busch. The presence of citrate was verified quantitatively by a colorimetric method. Radioactivity of the plated fractions was measured in a free flow gas counter. Under the influence of parathyroid extract, there was an increased amount of labeled citrate present. The mean results expressed as counts per minute per milligram DNA were 862 for the control group and 2,015 for the treated group. This increased citrate was available to form soluble chelates with calcium, withdrawing this cation from bone salts and from its protein-bound complex. Thus, the dissolution of the organized structure of bone may have been effected through cellular metabolism.

93. Relation of Fluoride Content of Human Bone to Its Chemical Composition.—*I. Zipkin, F. J. McClure, W. A. Lee, and N. C. Leone, National Institute of Dental Research, U. S. Department of Health, Education and Welfare, Bethesda.* Autopsy specimens of the iliac crest, rib, and vertebra were obtained from 23 individuals, 26 to 90 years of age (avg. 65.3), having a history of at least 10 years of exposure (avg. 29.9) to drinking water containing $<$1.0 ppm F to 4.0 ppm F. On an ash basis, the mean fluoride in the bones varied from 0.09 to 0.73 per cent and correlated well with the water fluoride. Where constituents of the bone ash were related to the increase in bone fluoride, all three bones varied consistently. For example, the iliac crest showed a significant increase in ash (53.5 to 58.6 per cent) and in Mg (0.50 to 0.57 per cent). Similarly, a significant decrease was seen in carbonate (5.83 to

5.20 per cent) and citrate concentration (2.23 to 1.56 per cent). No significant changes were seen in the Ca, P, Na, and K concentration in the bone ash of the iliac crest, rib, or vertebra.

94. THE COMPARATIVE METABOLISM OF Ca AND Sr IN THE RAT.—*A. S. Posner, Research Division, National Bureau of Standards, and R. C. Likins, M. L. Kunde, and D. C. Craven, National Institute of Dental Research, National Institutes of Health, Education and Welfare, Bethesda.* This is a report on the comparative metabolism of Ca and Sr in the rat. The appearance of Ca^{45} and Sr^{89} in the rat tibia, dentin, plasma, and urine after injection was observed by periodic sacrifice from experimental groups. Parallel experiments on synthetic hydroxyapatites tagged with Ca^{45} and Sr^{89} gave evidence that the preferential excretion of Sr^{89}, as compared to Ca^{45}, from the body of the rat is regulated on the level of the bone crystals as well as controlled by kidney and gut function. The preferential loss of Sr^{89} as compared to Ca^{45} on crystalline level might have been due to fractional crystallization.

95. THE VOID SPACE OF PACKED HYDROXYAPATITE CRYSTALS.—*Howard M. Myers, University of California, San Francisco.* The volume of the void between packed apatite crystals was determined under varying degrees of packing produced by centrifugation. The degree of packing can be expressed as the ratio of volume of void to the volume of void plus solid. This ratio, called the porosity, varied from 0.84 to 0.67 with increasing centrifugation. If the apparent binding of liquids by apatite is due to capillary attraction between the crystals, the volume of the liquids retained should be equal to the volume of the void space and to each other as well. The volume of different liquids retained by apatite packed to the same extent varied, however. The variation was predicted by the Stokes Equation and was due to the differing viscosities and densities of the liquids employed. In the case of water, more liquid was retained than predicted by the Stokes Equation. This excess was equal to 17.2 mM. per gram of apatite. The apatite crystals when dried and scattered over a wide area (to eliminate capillary condensation between crystals) adsorbed 18 mM. of water per gram. Surface calcium ions played only a small role in the adsorption of the water molecules since replacement of calcium by divalent cations with polarizabilities, varying by a factor of 300, resulted in only slight changes in water adsorption by the apatite crystals.

96. EFFECT OF SODIUM FLUORIDE ON CALCIUM SALTS AND DENTAL ENAMEL.—*Otto R. Trautz, Emma Fessenden, and Racquel R. Zapanta, New York University College of Dentistry, New York.* It is known that treating dental enamel with NaF solution reduces its solubility in acid, assumedly due to a change of the OH apatite to the less soluble F apatite. The simultaneous formation of crystallized CaF_2 (fluorite) is assumed to be due to the decomposition of the apatite. The purpose of this study was to obtain a broader experimental basis for the explanation of the NaF effect on enamel. Various calcium salts were immersed in 2 per cent NaF solution, usually for a period of 1 month, and then examined in the x-ray diffractometer for presence and amount of CaF_2. The immersed specimens were either mineral slabs with a flat surface, or flat plaques (1 by 2 cm.) formed from powders bonded with a small amount of ethyl cellulose. We observed very strong CaF_2 formation with calcite, monetite, brushite, and whitlockite (synthetic); medium-strong CaF_2 formation with Ca phosphate precipitate (Ca/P = 1.50); weak formation with OH apatite (precipitated and ignited, Ca/P = 1.67) and human enamel; a possible trace of formation with OH apatite (mineral); and no

formation with F apatite, francolite, and magnesian whitlockite. It was concluded that the foremost action of NaF upon dental enamel is the replacement of accessible carbonate and secondary phosphate by the acid resistant CaF_2. This removes the most soluble components from the enamel. Furthermore, F^- may replace OH^- in the surface of the apatite crystals. The decomposition of well-crystallized OH apatite to yield Ca for CaF_2 formation is comparatively slow and of secondary importance. F apatite seems not to be attacked by NaF.

97. A Possible Mechanism for the Formation of Beta-Tricalcium Phosphate During the Ignition of Dental Enamel.—*Gunter Kuhl and William H. Nebergall, Indiana University, Bloomington.* X-ray diffraction patterns of samples of powdered human dental enamel, which had been ignited in a muffle furnace at 600° C. for 1 week, were found to contain the more prominent diffraction lines characteristic of beta-tricalcium phosphate as well as those of hydroxyapatite. These observations led to experiments designed to show a possible mechanism for the formation of the β-$Ca_3(PO_4)_2$ during the ignition of dental enamel. It was found in the course of these investigations that a two-to-one molar mixture of pure anhydrous calcium monohydrogen phosphate, $CaHPO_4$, and pure calcite, $CaCO_3$, began to react at 600° C., giving β-$Ca_3(PO_4)_2$. When the ignition was carried out at 400° C., the $CaHPO_4$ was converted to γ-$Ca_2P_2O_7$ and the $CaCO_3$ remained unchanged. This means that the β-$Ca_3(PO_4)_2$ obtained at 600° C. was probably formed by the reaction: γ-$Ca_2P_2O_7 + CaO \rightarrow \beta$-$Ca_3(PO_4)_2$. These studies suggest that, in addition to hydroxyapatite, dental enamel may contain calcium monohydrogen phosphate and calcite, which react upon ignition of the enamel to form β-$Ca_3(PO_4)_2$. Calculations based upon the Ca^{++}, $PO_4^{\equiv}$, and $CO_3^{=}$ content of dental enamel showed that an approximate two-to-one molar ratio of $CaHPO_4$ to $CaCO_3$ is possible.

98. Structural Changes in Teeth and Their Supporting Structures Containing Bone-Seeking Radioisotopes.*—*Webster S. S. Jee and James S. Arnold, Department of Anatomy, University of Utah College of Medicine, Salt Lake City.* Teeth and bone changes in young adult beagles containing body burdens of bone-seeking radioisotopes, ranging between 0.0143 to 2.5 μc per kilogram (plutonium, radium, radiothorium, and mesothorium) and 0.143 to 25.0 μc per kilogram (strontium-90) administered in a single intravenous dose were studied using celloidin, radiographic, and autoradiographic technics. The plutonium and radiothorium deposited on mineralized surfaces exposed to blood supply such as the internal dentine surface beneath the predentine, the external cementum surface, and all bone surfaces. Radium, mesothorium, and strontium-90 deposited intensely on newly formed mineralized dentine, cementum, and bone, and diffusely in the remainder of the mineralized tissues. The lesions induced by these isotopes included formation of secondary dentine, death of cementocytes, resorption of cementum, dentine, and alveolar bone, damage of the periodontal ligament, fusion of bone to teeth (ankylosis), and replacement of dentine by haversian system-like bone and loss of teeth. No tumor was observed arising from teeth, but a few osteogenic sarcomas occurred in the mandible and maxilla. It was interpreted from the study of the skeleton from serial-sacrificed plutonium dogs that the damage was the result of both direct and indirect (damage to blood vessels supplying teeth and bone) irradiation effects.

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X. Electron Microscopy. March 20, Evening, Symposium I.

S1. Electron Microscopy of the Enamel Organ of the Rats' Incisors.*—*Edward J. Reith, Department of Anatomy, New York University.* Enamel organs of rats' incisors were prepared for electron microscopic examination. They were fixed in 1 per cent osmium tetroxide, dehydrated, and embedded in methacrylate. Thin sections were cut with a Porter-Blum microtome fitted with glass knives. Near the erupted end of the tooth, the ameloblasts were filled with yellow pigment, visible with the light microscope. Electron micrographs of this region showed a continuous basement membrane between the epithelial cells of the enamel ridges and the neighboring connective tissue. The cytoplasm of the cells, which made up the ridges, contained mitochondria, vesicles, and small granules. The ameloblasts contained large aggregates of dense granules. Serial thick sections indicated that they were yellow. Occasionally, fewer granules appeared in the center of an aggregate, in which case the arrangement of granules appeared in the form of a ring. The dense granules were also scattered singly throughout the cytoplasm. A small amount of space existed between the ameloblasts, except where they "contacted" each other; microvilli projected into this space. More apically, where the ameloblasts contained a minimum of granules, or no granules, there was more space between the cells. Microvilli also projected into this space.

S2. The Electron Microscopy of the Developing Amelodentinal Junction in the Molars of Syrian Hamsters.—*Mervyn B. Quigley, University of Alabama Medical Center, Birmingham.* Electron micrographs were made of developing molar teeth of newborn hamsters. Fixation was in buffered osmic acid (pH 7.4). The tissues were embedded in methacrylate, and sectioned on a Porter-Blum microtome at 200 to 300 Å. No shadowing or decalcification was carried out. The first indication of the amelodentinal junction was a very delicate interface between the enamel epithelium and the underlying embryonic connective tissue. A fibrous barrier, the amelodentinal membrane, then formed on the dentinal side of the interface, before the epithelial cells had become columnar. The odontoblasts then differentiated and were separated from the membrane by an incomplete double structure which has been tentatively identified with the so-called terminal bar apparatus. The forming ameloblasts were separated from the amelodentinal membrane by an incomplete double membrane, which became discontinuous and later disappeared, so that no evidence of the original interface remained. The amelodentinal membrane became invaded from the odontoblast side by dark osmiophilic material. Fibers with the typical periodicity of collagen could be seen transversing what was now predentin and the amelodentinal membrane. The course of these fibers was somewhat irregular, and they occurred in groups of one or two or in larger groups up to eight or ten. Finally, as enamel matrix was formed, the membrane became extremely electron-dense, and the fibrillar nature of the membrane was obscured.

S3. Preliminary Electron-Microscopic Observations on Carious Dentine.—*Erling Johansen and Harold F. Parks, University of Rochester School of Medicine and Dentistry, Rochester.* Soft carious dentine from freshly extracted human molars was excavated and prepared for electron-microscopic examination of ultra-thin (silver) sections. Microorganisms, which were present in large numbers, were thickly packed in the lumina of the dentinal tubules; they

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were also situated within recesses extending from tubules into the intertubular matrix. A layer of rather densely mineralized material, irregular in thickness and discontinuous in distribution, formed a sort of lining of many dentinal tubules. In its thickest parts, this layer was seen to be trilaminar, consisting of a thick middle layer containing relatively loosely packed, randomly oriented large crystals, and thin inner and outer layers consisting largely of densely packed smaller crystals, apparently oriented parallel to one another. The thinner parts of this lining were mainly composed of the smaller, tightly packed crystals. Treatment of sections with phosphotungstic acid demineralized the lining, leaving a nondescript residue, but failed to reveal the presence of recognizable collagenous fibrils. In untreated sections, the dentinal substance proper exhibited a relatively sparse distribution of crystals disposed individually or in small clusters, and having no apparent preferred orientation. Phosphotungstic acid treatment removed the crystals and simultaneously revealed, by its staining action, an abundance of compactly arranged collagenous fibrils, running mainly parallel to one another.

S4. Electron Microscopy of Heavy Metal Deposition in Living Hard Tissue.—*Robert M. Frank, and Reidar F. Sognnaes, Harvard School of Dental Medicine, Boston.* The uptake of various ions in bones and teeth has mainly been studied by means of radioactive isotopes and autoradiography. With the high resolution afforded by the electron microscope, it seemed of interest to attempt a direct visualization of penetrating ions by in vivo ingestion of heavy metal salts in solution and then capitalizing on the electron-dense property of whatever metal salt might become deposited within minute regions of the ultrastructural pattern of bones and teeth. In the present study, albino rats received drinking water containing 0.1 per cent of either silver nitrate or gold chloride for periods of up to a year. Small blocks of teeth and maxillary bone were fixed in buffered osmic mixture and in neutral formalin, respectively. Some specimens were decalcified; others were directly embedded in methacrylate. Ultra-thin sections of decalcified and nondecalcified material were cut with the Servall-Porter microtome, using a glass or diamond knife, and examined with an electron microscope, RCA model EMU-3B. Histologic sections were also prepared for optical microscopic controls. Grossly and by ordinary histologic examination of ground sections, it was found that the rats fed silver nitrate solution had a much deeper penetration of the tissues than did the rats receiving gold chloride. The gold chloride caused a superficial deposition in the coronal part of the teeth as though they had been covered with gold foil. Silver nitrate, on the other hand, in addition to causing blackening of the surface, showed deep penetration into the enamel as well as the dentin. In the electron micrographs, deposits were in the form of extremely dense particles with sharp outlines and varied from aggregates of a few microns in diameter to very small submicroscopical granules in enamel, dentin, and jaw bone.

S5. Ultrastructural Changes in Dentine Occurring in Incipient Caries.*—*C. Gordon Hewes, Ralph R. Steinman, and Robert W. Woods, Departments of Anatomy, Prosthodontia, and Biophysics, College of Medical Evangelists, Loma Linda, Calif.* Changes occurred in dentine within 7 days after weanling rats were placed on a high sucrose diet. Differences in dye concentrations were noted as a result of injecting trypan blue intraperitoneally into rats fed a high-sucrose diet and those fed Purina laboratory chow. The dentine of teeth of animals fed a high-sucrose diet appeared less discolored than that of teeth of animals fed Purina laboratory chow. To determine the cause

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of this difference, an electron microscopic study was made of the dentine of teeth from young animals of each diet group without giving trypan blue injections. Molar teeth were extracted, root tips removed, and the teeth fixed in osmium tetroxide. It was possible to section both the dentine and enamel of these teeth without demineralization. Such procedure permitted observation of effects of diets occurring in the teeth immediately preceding sacrifice without the inevitable changes resulting from the demineralizing process. Electron micrographs demonstrated that the odontoblastic processes of molar teeth of sucrose-fed rats appeared to be patent tube-like structures in contrast to the nearly closed or solid protoplasmic processes in dentine of teeth of animals fed Purina laboratory chow.

S6. SUCROSE RETARDATION OF ACID ETCHING IN DENTAL ENAMEL: AN ELECTRON MICROSCOPY STUDY.—*R. Quentin Blackwell, L. McMillan, and L. S. Fosdick, Northwestern University Dental School, Chicago.* It has been previously shown that the solubility of powdered human dental enamel in acid solutions is reduced in the presence of sucrose. It was thought of interest to determine whether or not this phenomenon could be observed in sound tooth enamel. Sound tooth enamel surfaces were flattened by means of an abrasive wheel and polished with calcium carbonate in order to prepare a standard flat surface. These smooth standardized surfaces were then treated in the following manner. One half of the surface was covered by means of pressure-sensitive tape and the exposed half was etched for 15 minutes by one of the test solutions. The etched surface was washed with water, dried and covered with pressure-sensitive tape, after which the half that had been covered with tape was exposed. This exposed surface was etched for 15 minutes with another test solution, after which it was washed and dried. The tape was removed and the boundary between the two halves was replicated. The replicas were placed upon wire screens, shadowed with chromium, and observed in the electron microscope. The solutions used were 0.1 M lactate buffer, pH 4.5; 0.1 M lactate buffer containing 1 M sucrose solution, pH 4.5; 0.1 M lactate buffer containing 1 M sodium chloride, pH 4.5; and 1 M sucrose solution, pH 7. In each case the effects of the lactate buffer containing the additional substance was compared with that of the pure lactate buffer. In each case, the surface exposed to the 0.1 M lactate buffer, pH 4.5, was markedly etched, while those solutions containing the 1 M sucrose showed only slight etch. The lactate buffer containing the sodium chloride produced an etch comparable with lactate buffer alone and the 1 M sucrose, pH 7, solution produced no etch. From these results it would seem that sucrose in acid solutions causes less solution of intact enamel than do similar acid solutions without the sucrose.

S7. STUDY OF EMULSIFIED PERIDENTAL MEMBRANE WITH THE ELECTRON MICROSCOPE.—*D. Y. Burrill, L. M. McMillan, and L. S. Fosdick, Northwestern University Dental School, Chicago.* In March, 1958, McMillan reported finding structures which were apparently collagen fibers or their remnants in exudate from peridental pockets. Some of the fibers appeared partially degenerated. Because of the theoretical interest of this finding, a method of emulsifying peridental membrane fragments was devised. A freshly extracted tooth was carefully washed in distilled water and a small amount of adherent peridental membrane was removed from a selected location on the root. The fragment was placed in about 1 ml. of distilled water in a short test tube about 17 mm. in diameter. A stainless steel blade, about 13 mm. in diameter, was affixed to a mandrel so that it could be immersed in the water in the test tube and was rotated there at about 20,000 r.p.m. At this speed it took about 30 minutes to break up most of the peridental membrane. After a few minutes of

operation, the water took on an opalescent appearance. At the end of the run, small amounts of the emulsion were placed on Formvar-coated screens, dried, shadowed with chromium, and viewed and photographed with the electron microscope. The emulsion was found to contain large numbers of particles which seemed to be fibers of the same structure as those tentatively identified as collagen in the studies of the exudate. It is believed that this method may be useful in studying the degeneration of the peridental membrane.

S8. A Comparison of Caries-Immune and Caries-Susceptible Dental Plaques by Means of the Electron Microscope.—*L. McMillan, D. Y. Burrill, and L. S. Fosdick, Northwestern University Dental School, Chicago.* Dental plaque was secured by harvesting from between the teeth with dental floss. The plaque was placed in water and homogenized mechanically. The homogenate was diluted to suitable concentration and portions transferred to previously prepared Formvar-coated screens and dried. After shadowing, the specimens were viewed and photographed with the electron microscope. It was possible to distinguish clearly many rods, cocci, spiral, and filamentous forms. Many of the rod forms appeared as empty or partly empty cells, which may indicate that the organisms were nonviable before the preparation was made. This method offers a possible useful means of studying the flora and fauna of the mouth for correlation with clinical findings.

XI. Bone Implants. March 20, Evening, Symposium II.

S9. Further Observations on Bone Isografts Within Diffusion Chambers.—*Paul Goldhaber, Harvard School of Dental Medicine, Boston.* In a recent study concerning the fate of bone isografts within "diffusion chambers" implanted subcutaneously into mice, it was found that growth and differentiation of cells into a bone-like tissue occurred within 8 weeks, primarily on the inner surface of the Millipore Microweb filters. Since these specimens had been decalcified, there was no opportunity to determine the presence of calcium in the new "bone." In order to clarify this point, the experiment was repeated using 10 diffusion chambers, each containing a 2-day-old mouse calvarium. After 2 months, the chambers were removed and the implants (with attached filter) were sectioned and stained without decalcification. Routine hematoxylin and eosin-stained slides revealed the same pattern of new bone formation as in the previous experiment. Of greatest interest was the finding of a thin, dark-staining acellular zone (continuous with the new bone on the surface of the filter) that extended about 20 to 30 μ *into the filter.* At the innermost edge of the zone were small, dark-staining globules. By means of phase-contrast microscopy, it could be seen that the width of the zone appeared to be related to the distance that a precipitate (possibly cytoplasmic processes from the surface cells) extended into the porous filter. The new bone on the surface of the filter, as well as the acellular zone and globules *within* the filter, appeared calcified, as demonstrated by microradiography as well as by the von Kossa stain. The presence of collagen and other bone matrix components (presumably mucopolysaccharides), prior to calcification, suggests that the acellular zone within the filter is a direct extension from the new cellular bone on the surface of the filter, having essentially the same histologic properties of true bone except for the exclusion of osteocytes.

S10. Healing of Experimentally Produced Fractures in Dogs.—*Henry B. Clark, Jr., and Paul A. Hayes, University of Minnesota School of Dentistry,*

Minneapolis. Surgical fractures of the body of the mandible were produced in an edentulous area in 16 mongrel dogs. Eight were secured with direct bone wiring and 8 were plated. A template with four holes in a straight line was laid across the fracture line as a guide for placement of two small bur holes on each side. These holes were filled with silver amalgam to permit later radiographic recording of amount of mobility. In each case, the amount of mobility was recorded rediographically at weekly intervals by taking two radiographs under anesthesia, one with a 10-pound upward pull on the anterior segment and one with a 10-pound downward pull. Tracings of the radiographs were made to record the position of the amalgam dots at the time of the upward and downward forces. Lines were drawn through the dots, tracings superimposed, and amount of angulation read in degrees. All animals were sacrificed at 6 weeks, the fixation appliances were removed, and a final set of radiographs made. The wired animals showed more mobility throughout than the plated animals, but there was a sharp drop in amount of movement at the 4- to 5-week point. In all cases, the amount of mobility at the time of sacrifice was the same before and after removal of the plates and wires. The amount of mobility of the wired animals at the time of sacrifice was 2 to 3 times greater than that of the plated animals.

S11. EFFECT OF OSTEOGEN IN EXTRACTION SOCKETS OF RATS.—*Bryce A. Gilbertson, and Henry B. Clark, Jr., University of Minnesota School of Dentistry, Minneapolis.* Lower right and left first molars were extracted from 36 white rats. Cultured calf bone paste (Osteogen) was placed in the socket on the left with the right side serving as control. The animals were sacrificed at 16 hours and 1, 2, 4, 6, 8, 10, 12, 15, 20, 25, and 30 days after surgery. The mandibles were fixed in 10 per cent formalin, decalcified in formic acid, embedded in paraffin, sectioned in the frontal plane and stained with hematoxylin and eosin. Sections were cut at 7 μ. The control sockets showed minimal inflammatory response and no foreign body reaction. Epithelium covered the socket openings by the fourth day. New bone appeared on the sixth day and all sockets were filled with bone on the tenth day. The experimental sockets showed moderate inflammation through the fourth day and foreign body giant cells through the fifteenth day. Epithelization was complete on the eighth day. Some new bone appeared on the sixth day but the sockets were not filled until the thirtieth day. The earliest bone formation occurred in those portions of the sockets which contained no calf bone particles. The calf bone was eventually embraced by new bone growing out from the socket wall.

S12. USE OF ANORGANIC BONE TO INCREASE RIDGE HEIGHT IN DOG JAWS.—*Roger J. Burke and Henry B. Clark, Jr., University of Minneosta School of Dentistry, Minneapolis.* Heterogenous anorganic bovine bone grafts were placed in partially edentulous dog jaws to increase mandibular ridge height and to study the response of oral tissue to anorganic bone. Contoured pieces of this material were implanted in previously prepared maxillary and mandibular edentulous alveolar ridge sites. By using 8 dogs, 24 graft beds and 8 control sites were made available for study. Fifteen implants were placed in prepared mandibular medullary bone (inlay) defects, while 17 implants were laid over maxillary cortical bone (onlays) and covered with mucoperiosteum. The implants were observed grossly and x-rayed at various intervals during the 26-week period. The animals were sacrificed at periods of 10 days to 26 weeks. Fourteen of 17 onlays and three of 15 inlays remained in a healthy state until the time of sacrifice. The remainder of the implants

were lost due to mucosal dehiscence or wound infection. Microscopic study of the grossly successful implants showed minimal foreign body reaction, satisfactory acceptance, and marked osteogenic response of the oral tissues to the bone. Alveolar ridge height was increased in one of the eight mandibular graft beds.

S13. Resorption Studies of Anorganic Bone.—*Emmett R. Costich and James R. Hayward, University of Michigan School of Dentistry, Ann Arbor.* Previous studies have shown unusually good host tolerance of anorganic bone implants. This is a disadvantage when using anorganic bone as a graft because the graft is not resorbed and replaced by host bone. A study was carried out using anorganic bone treated with 2 per cent gelatin solution, calcium hydroxide 2 per cent (2 grams of CaOH suspended in 100 c.c. of distilled water), and distilled water. Pellets of dry 20 to 40 mesh anorganic bovine femoral head were immersed in the solutions to draw the material into the macro- and micro-channels in the bone. Dry anorganic bone was the fourth material used in this study. The implant materials were autoclaved before use. Eighteen 4-month-old hamsters were divided into two groups. Group I was composed of 4 females and 8 male animals and received a single pellet of each type of bone preparation, subdermally in each quadrant of the back via a midline incision. The backs were shaved and washed before surgery. Group II consisted of 6 male animals. Bone was placed in each quadrant of the back in an approach similar to Group I except that the bone was placed in incisions made in muscle. This was done to compare response in relatively vascular and avascular situations. One animal in Group II died of peritonitis 12 days postoperatively. The animals were sacrificed at intervals over a period of 1 year. The bone was unchanged macroscopically, and histologic sections stained with hematoxylin and eosin, Masson's trichrome, and Mallory's trichrome showed no evidence of bone resorption or new bone formation. All specimens showed the anorganic bone enclosed in a capsule of fibrous connective tissue. The material used in the concentration described did not stimulate resorption of anorganic bone.

S14. Resorption Characteristics of Autogenous and Anorganic Bone Implants.—*William H. Bell, University of Texas Dental Branch, Houston.* Previous experiments have shown that anorganic bone implants in human tooth extraction sockets were very slow to be resorbed. The purpose of this experiment was to compare the rate of resorption of cancellous anorganic bone and cancellous autogenous bone implants. Anorganic and autogenous bone implants (obtained from iliac crest), 7 mm. in diameter and 2 mm. in thickness, were placed intermuscularly between the heads of the gastrocnemius muscle of 7 adult mongrel dogs. The operative sites were subsequently x-rayed every 2 weeks. Radiographs revealed that the autogenous bone implants were completely resorbed in an average of 6 weeks. None of the anorganic bone implants showed any detectable radiographic evidence of resorption. Three dogs were followed for a period of 36 weeks. The clinical success of a bone graft is partially dependent on rapid resorption of the graft material and replacement with new host osseous tissue. Because anorganic bone was very slow to be resorbed, it did not compare favorably with autogenous bone. These experimental findings were substantiated by histologic study of specimens removed from human healing tooth sockets which had been filled with autogenous or anorganic bone particles at the time of the tooth extraction. These studies revealed that anorganic bone particles were resorbed much slower than autogenous bone chips.

S15. LONG-TERM HISTOLOGIC RESPONSE TO IMPLANTS OF ANORGANIC HETEROGENOUS BONE IN MAN.—*Philip J. Boyne, Marine Corps Base, California, and Harvey W. Lyon, Dental Division, Naval Medical Research Institute, Bethesda.* While initial host acceptance of anorganic bone implants has been demonstrated previously, the ultimate fate of these osseous grafts has not been observed. A long-term histologic evaluation of anorganic bone implants in man was undertaken to parallel similar animal studies currently being undertaken. Implants of anorganic bovine bone were placed in oral bony defects of human patients. These bony defects had resulted from the destruction of alveolar bone by the commonly occurring periapical and periodontal pathologic lesions and from loss of osseous structure attending complicated exodontia. The postoperative course was uniformly uneventful. Biopsies of the implanted defects were obtained postoperatively in 15 cases, ranging from 3 to 18 months. Histologic evaluation of these specimens revealed a general lack of any significant inflammatory response surrounding the anorganic particles. At the periphery of the defects, the anorganic particles were being remodeled by reactive-appositional bone growth. There was a general lack of osteogenic activity around anorganic particles which were not in intimate contact with the host bone. Implanted particles lying immediately beneath the mucoperiosteum or in the central portion of the defect were, in general, surrounded by fibrous connective tissue. In some defects, in which attempts had been made to restore alveolar contour and height, the labial and buccal cortices had re-formed, engulfing anorganic particles in the newly formed matrix. Osteoblastic and osteoclastic activity at the periphery of these particles, however, was minimal. This persistence of implanted anorganic particles over prolonged periods of time postoperatively, and the general long-term histologic status of these implants, would tend to indicate the limitations in the clinical use of this material.

S16. HOST RESPONSE TO IMPLANTS OF HETEROGENOUS ANORGANIC BONE.—*Harvey W. Lyon, Carl A. Ostrom, and Fred L. Losee, Naval Medical Research Institute, Bethesda, Lloyd A. Hurley, New York Orthopaedic Hospital, New York, and Philip J. Boyne, Marine Corps Base, California.* Previous short-term studies have shown that heterogenous anorganic bone implants within an osseous bed satisfy two major criteria for a successful transplant, namely: lack of foreign body reaction and subsequent bony union with the host. The following long-term studies are in process to evaluate whether the third criterion, functional reconstruction of the graft, occurs within anorganic bone implant sites. Within a 2-year period, anorganic bone has been placed in 140 maxillofacial bony defects in 10 adult Rhesus monkeys and 42 mongrel dogs, 54 pulpal applications in 42 dogs, 20 lumbar and 20 lumbo-sacral spine fusions in 40 dogs, 40 long-bone defects in 20 dogs, and 69 maxillofacial and long-bone implants in human patients. All preoperative implant sites were free of any apparent infection or visible inflammatory process. Observations at 30, 60, 90 days and 1 year postoperatively, indicate that the rate and extent of remodeling in these implants varied with the location, preparation, and size of the implant site. Implants placed in contact with exposed, bleeding marrow-vascular spaces in long bones and vertebral defects exhibited a greater degree of remodeling than similar implants placed within the relatively avascular walls of sockets and other extraction defects. Residual, trapped, unresorbed anorganic cortical or spongy chips appeared to be disintegrating in healed, 1-year postoperative, maxillofacial graft sites in adult Rhesus monkeys. Response of pulpal tissues to 200/325 mesh anorganic bone or dentine was similar to calcium hydroxide controls.

XII. Hatton Award Competition II. March 21, Forenoon, Section A.

99. Mitotic Activity of Human Oral Epithelium When Exposed to Hydrogen Peroxide.—*Anthony W. Gargiulo, Loyola University, Chicago.* This study was concerned with the effect of hydrogen peroxide upon human attached gingival epithelium when subjected to prolonged exposure. The morphology, mitotic index, mitosis patterns, cell density, and their possible alterations were studied. Two age groups were used, a young age (25 years) and an old age (56 years). Each of the groups was further subdivided into a control and experimental group. The experimental specimens were exposed to tri-weekly applications of 30 per cent hydrogen peroxide for a period of 30 days. Following fixation and sectioning, random samples of tissue were selected. Tracings of magnified (× 100) projections of each section were made. From these tracings, we were able to measure the total surface area of the specimen and ascertain the estimated total cell population. From this, mitotic index and cell density were recorded. In addition, each specimen was examined for the thickness of the keratin layer, and suprapapillary and basal cell layer heights. The average mitotic index increased in the experimental groups; in the young from 0.79 to 6.50, and in the old from 1.69 to 8.76. The over-all cell density in the experimental groups was decreased, but was always slightly higher in the older age groups. Upon observing the various phases of mitosis, it was seen that the experimental groups had a preponderance of mitotic figures in prophase, whereas the control groups had a rather even distribution in all phases. In the experimental groups, the thickness of the keratin layer and suprapapillary ridge were seen to increase, and the size of the basal cells was also increased.

100. Observations on the Morphologic Alterations of Rat Mast Cell Granules.—*Daniel W. Mitziga, Zoller Dental Clinic, The University of Chicago, Chicago.* Recognition of the connective tissue mast cell has existed for approximately 100 years, but only within the past 10 to 15 years have some of its chemical constituents been elucidated. The purpose of this investigation was to isolate the mast cell granules by the method of differential centrifugation and to perform chemical assays, as well as to observe morphologic characteristics of the mast cell granules in all phases of isolation. Female white rats (Sprague-Dawley), 3 months of age and weighing 150 to 225 grams, were used as experimental animals. Preliminary surveys of rat tissue revealed highest proportions of mast cells in the tail, snout, tongue, gingivae, and subcutaneous tissue of the back and feet. Due to separation difficulties with stromal cells, subcutaneous tissue from back and feet were selected for experimental purposes. Light and electron photomicrographs indicated that rat mast cell granules swelled irregularly when spilled into tissue, and that certain media produced alterations in the granules such that fragmentation and dispersion of the metachromatic material occurred even in concentrations as low as 0.15 M. Recognition of morphologic alteration is important and should be considered whenever isolation of cell particulates is contemplated for purposes of chemical assay.

101. Application of Fluorescent Antibody Technics to Gingival Tissues.—*Burton Rosan, University of Pennsylvania, Philadelphia.* A strain of *Streptococcus mitis* which produced hyaluronidases was isolated from subgingival scrapings of human patients with periodontal disease. The fraction of the culture filtrate showing hyaluronidase activity was isolated from the culture medium and used as an antigen in rabbits. The serum gamma globulin

fraction, obtained after the injections, was conjugated with fluorescein isocyanate (referred to as labeled gamma globulin) according to the method of Coons. Biopsies of gingival tissues from human patients with periodontal disease were prepared in serial sections. Staining was accomplished by adding a drop of labeled gamma globulin to the tissue sections in a moist chamber and incubating for 15 minutes at room temperature. Control sections were exposed to unlabeled gamma globulin to block reactive sites, before being exposed to labeled gamma globulin. Other control sections were stained with hematoxylin and eosin to allow for conventional orientation of the specimen. Examination of all sections was made with the fluorescence microscope. Preliminary observations indicated presence of streptococcal antigens (presumably hyaluronidases) in the connective tissues slightly beyond the site of greatest inflammatory infiltration. The connective tissues farther away from the inflammatory processes and still containing intact gingival fibers were not stained with the labeled gamma globulin.

102. ORIGIN OF THE EPITHELIAL ROOT SHEATH.*—*Franz W. Logan, Major, USAF (DC),** Ohio State University, Columbus.* Development of the epithelial root sheath was studied in first molars of 24 Sherman albino rats. Specimens of age range, 2 through 45 days, were prepared for histologic examination. Serial sections were cut in longitudinal and transverse planes and stained with hematoxylin and eosin. The cervix of the rat molar was established by a dentine eminence. Root formation began after the eminence had formed. It could not be determined by morphology of the cells of the epithelial diaphragm or dental papilla. A growth center at the fundus of invaginating primordial ectoderm gave rise to the dental epithelium. An epithelial diaphragm, partially enclosing the base of the dental papilla, was established from daughter cells of the fundic growth center. The epithelial root sheath was the next cytomorphic stage of radicular dental epithelium. It was a double layer of cells adjacent to new-formed predentin, and survived until calcification began. The root sheath was continuous with enamel organ elements only at the start of root formation. There was an axial movement of predentin and contiguous cell layers before the peripheral dentine form was established.

103. SUBMAXILLARY SALIVA AS A CULTURE MEDIUM.—*Benjamin F. Hammond, University of Pennsylvania, Philadelphia.* Previous studies indicated that parotid saliva supports the growth of several oral microbes. Thus, it seemed appropriate to investigate submaxillary-sublingual saliva as the sole source of nutrient for microorganisms. The saliva was collected by means of sterile aluminum trays adapted to the submaxillary mucosa (a modification of the Schneyer apparatus) and subsequently sterilized in thin films by ultraviolet light. The method of sterilization adopted did not alter the enzymatic properties of the saliva, using amylolytic activity as an index. The method did not result in a significant rise in H_2O_2 concentration, a factor which might limit its use in cases where H_2O_2-sensitive organisms were studied. Growth curves of (A) *Bacillus subtilis* var. *niger*, (B) *Aerobacter cloacae*, (C) *Lactobacillus casei*, (D) *Streptococcus faecalis* and (E) a yeast isolated from the oral cavity were obtained by colony counts from separate roller tubes (using a standard inoculum) after 0, 24, 48, and 72 hours of incubation at 37° C.

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**Assigned as graduate student, Ohio State University, Columbus, Ohio.

All saliva samples were buffered to pH 6.8 with M/15 phosphate and appropriate controls were used for each growth curve. The results showed that cells of A multiplied and spores germinated but at a slower rate than the nutrient control. Microbes B and E remained viable but with no significant increase in cell numbers for 72 hours. Organisms C and D showed a gradual loss of viability over the incubation period. These experiments indicated that human, sterile submaxillary-sublingual saliva contains nutrients for maintenance of viability but, except for organism A, these were far below the levels of an optimal medium. These results are similar to those found in work with parotid saliva for organisms A and E, but more nutrient is available than in parotid saliva for bacterial populations of B, C, and D.

104. An Apparatus for Cutting Thin Serial Sections of Undecalcified Teeth.—*Barrie Gillings, Eastman Dental Dispensary, Rochester.* An inexpensive, easily constructed apparatus for the rapid, routine preparation of thin plano-parallel serial sections of undecalcified tooth structure is described. Plastic embedded specimens measuring up to 50 mm. by 35 mm. by 15 mm. can be serially sectioned. The apparatus uses a 4 inch diameter diamond disc rotating at 6,500 r.p.m. in conjunction with a precision specimen transport. Plano-parallel sections of dentine, cementum, and calculus of between 40 to 50 μ may be obtained routinely, and thinner sections are possible using greater care. Specimens containing enamel or restorations can be sectioned routinely at 100 to 120 μ. Although thinner sections may be cut, they are more prone to damage when handled. The apparatus is semi-automatic and can produce a section every 5 to 7 minutes, with approximately 25 sections obtainable from 1 cm. of material. Individual sections have a polished surface and, for most studies, would require no further preparation. The average thickness of individual sections, determined by measuring several different areas on the surface, indicates a uniformity within $\pm$ 2 μ, while the mean deviation of the average thicknesses of serial sections is $\pm$ 3 μ for dentine specimens and $\pm$ 7 μ for sections containing enamel.

105. The Dentino-Pulpal Membrane.—*Rudy Chris Melfi, The Ohio State University, Columbus.* It is generally conceded that the contents of the dentinal tubules are direct processes of the odontoblasts. This report established a relationship between the cell body of the odontoblasts, a dentino-pulpal membrane and the tubular content. Teeth of human beings, rats, and dogs were studied. Of a total of 184 specimens, 82 human teeth were examined histologically. Approximately half of each group of specimens were taken during active dentinogensis. The remainder consisted of adult specimens. The sections were stained with hematoxylin and eosin, Mallory, Masson, Foote and Wilder silver, van Giesen, Verhoeff, and aniline blue, by means of various histochemical methods. The dentino-pulpal membrane could be interpreted as a primitive increment of growth during dentinogenesis. The dentino-pulpal membrane consisted of Korff's fibers and short, blunt processes of the odontoblasts. The membrane gave rise to extensions around which dentine formed. The enzymes and various substrates associated with calcification were also located in the dentino-pulpal membrane.

106. Physiologic Response to Dental Stress.—*Irwin I. Ship, National Institute of Dental Research, U. S. Department of Health, Education and Welfare, Bethesda.* The response of the eosinophilic neutrophil in the circulating blood has been used in many studies as a convenient yardstick of the body's reaction to stress. It has been shown that decrease in the number of eosinophils is associated with increased adrenocortical activity that accompanies

a state of stress, and the magnitude of the decrease is proportional to the degree of adrenal response. Twenty-four healthy adult individuals with extensive caries attended a total of 235 dental appointments each 45 minutes in duration. Prior to, and 4 hours after each appointment, eosinophil counts were obtained on capillary blood taken by standard finger puncture into 3 standarized white cell pipettes, diluted 1:10 with 0.1 per cent phyoxine in 50 per cent propylene glycol, and read within 4 hours in six standardized counting chambers. Appointments were as follows: (1) control (no procedures performed, patient rested alone in dental chair for 45 minutes), (2) examination and interview, (3) x-ray examination, (4) prophylaxis, (5) operative dentistry, consisting of amalgam restorations, using 15,000 r.p.m. dental engine, (6) polishing of restorations, and (7) oral surgery. In categories 5 and 7, work proceeded from tooth 1 to 32 in sequence. Blind studies utilizing 50 mg. of pentobarbital taken ½ hour prior to appointment were compared with lactose placebo or nothing. Two per cent Lidocaine local anesthesia was compared with physiologic saline or nothing. The schema was prepared by a statistician, and neither patient nor operator was informed. One operator performed all procedures in the same dental chair. Results showed that variation between individuals was greater than within individuals, and variation between treatments was greater than within treatments. Adrenocortical activity varied directly with the magnitude of stress involved in the dental procedure, with all procedures showing significant differences from control appointments. The level of activity was not affected by local anesthesia or barbiturate premedication in all categories except oral surgery, in which barbiturate made a profound change in the level of response. Differences in responses between treatments, except for oral surgery, were not significant, with few exceptions.

107. CONSIDERATION OF A TRADITIONALLY ACCEPTED CONCEPT OF MASTICATORY PHYSIOLOGY.—*Harold C. Askew, University of Alabama School of Dentistry, Birmingham.* Food distribution during mastication was determined for 61 subjects by radiographic observations during the mastication of a radiopaque bolus. Each subject had serviceable complete dentures (artificial) which he used regularly when eating and wore continuously during the day. Observations were made from a posteroanterior direction. The distribution was tabulated as unilateral (right or left), simultaneously bilateral, anterior or center, or a combination of these. One hundred and eight (40 per cent) of 273 observations by a static x-ray picture method showed a simultaneously bilateral food distribution. One hundred and sixty-five (60 per cent) showed a unilateral food distribution. Twelve subjects were examined both by static x-ray pictures and fluorography. Eight of the 12 (66.7 per cent) showed the same food distribution by both methods. Contrary to traditional thought, these results indicate that a substantial part of the denture population chews food on both sides of the mouth at the same time.

XIII. CARIES: HUMAN. MARCH 21, FORENOON, SECTION B.

108. STUDIES OF FOOD RETENTION ON TOOTH SURFACES.—*J. McCormick and J. F. Volker, University of Alabama School of Dentistry, Birmingham.* Food retention in occlusal pits, fissures, and interproximal areas has been investigated, using radioactive isotopes and chemical spot tests. To study food retention in vivo, radioisotopes were used with 2 to 15 μ of $RI^{131}SA$ being mixed with caramel and deposited in the test area. A small Geiger probe counter was fixed in a standardized fashion over the occlusal surface or interproximal area and counts made at intervals of 15 minutes. High counts were obtained

for periods up to 2 hours. To study caramel retention for longer periods, the triphenyltetrazolium chloride spot test for carbohydrates was employed on washings from specific areas of human teeth mounted on dentures. Samples were collected up to 11 hours after the subjects chewed the caramel. Carbohydrate was detected as long as 8 hours after eating in occlusal fissures. Interproximal retention could be demonstrated for 2 to 4 hours. These findings indicate that retention, especially in deep occlusal fissures, occurs for extended periods of time.

109. The Removal of Microscopic Particles From Human Teeth.—*Aage Jorgensen and Mervyn Quigley, University of Alabama School of Dentistry, Birmingham.* Baked goods, liberally tagged with iron carbonyl particles, were chewed by test subjects. The tagged baked goods were expectorated when subjects felt the urge to swallow. The teeth and mouth were then cleaned by conventional tooth brushing with a commercial paste, followed by rinsing with cold water. At intervals afterward sections of commercial gum were chewed for 5 minutes. Smears were made of the removed gum bolus, and contact microradiographs were taken with a Philips CMR unit. Iron was detected in gum samples chewed several hours after the tagged baked goods—tooth brushing procedure. In an attempt to quantitate the iron in the recovered chewing gum, a known amount of a standard solution of contaminated gum was plated on thin cellophane, and further micrographs made. This method has three potential uses. These include: (1) determination of the effect of various cleansing procedures, (2) evaluation of the detergent action of foodstuffs and gums, and (3) evaluation of the retentive nature of foodstuffs.

110. Centrifugal Technic for Measuring Food Retention.—*Donald J. Beck and Basil G. Bibby, Eastman Dental Dispensary, Rochester.* Investigation was directed toward developing an in vitro technic for measuring the retentivity of food around the teeth. The technic consists basically of loading a chewed test foodstuff onto teeth mounted in the caps of centrifuge tubes. After controlled centrifugation the teeth were removed from the caps, washed in standard volume of water, and the total carbohydrate in the washings determined, using a modified anthrone colorimetric technic. The major modifications are (a) the use of anthrone in ethyl acetate solution as reagent, and (b) controlled heating and cooling of the samples. From the total carbohydrate in the washings, and the predetermined carbohydrate content of the test foodstuff, the amount of food that adhered to the teeth was calculated. This value, in milligrams was taken as the centrifugal retention index of the food. Reproducible indexes for a group of foods were obtained. Highest retention values were obtained with candies (caramel and chocolate) and the lowest values with cereals (puffed rice and corn flakes) and fruits (canned pineapple and apple). The retention ratings of all foods tested showed some correlation with the ratings obtained with previously employed intra-oral technics. In an investigation of the effect of temperature on food retention, it was found that the centrifugal retention index decreased with increasing temperatures in the range 20° to 37° C.

111. Further Studies on the Effect of Carbohydrate Substrates on Plaque pH In Vivo.—*I. Kleinberg, Faculty of Dentistry, University of Manitoba, Winnipeg, Canada, and G. N. Jenkins, Medical School, King's College, Newcastle upon Tyne, England.* Earlier studies (Jenkins and Kleinberg, J. D. Res. 35: 964, 1956; Kleinberg, J. D. Res. 37: 756, 1958) were extended, using the antimony microelectrodes and procedure previously described (Kleinberg, Brit. D. J. 104: 197, 1958) to determine those properties of carbohydrate

substrates that influence plaque pH in vivo. The pH changes in (a) two dental plaques in each of three anterior regions of the mouth (maxillary labial gingival, maxillary interproximal, and mandibular interproximal) and (b) the parotid and mandibular salivas were studied simultaneously in each of 5 subjects before, during, and after each of 1 per cent and 20 per cent glucose rinses, glucose sweet, white bread, apple, and cooked rice; the retention of the more solid substrates was not allowed to occur. The lowest plaque pH minima were obtained with the 20 per cent glucose rinse, next with the glucose sweet, next with white bread, then apple, and the highest values with the 1 per cent rinse and the cooked rice. When bread and rice were retained, however, sufficient substrate from these foodstuffs appeared to be available to the plaque bacteria so that the plaque pH minima could be reached. In further experiments, jam plus white bread when retained gave the same pH minima as did retained bread without jam, again indicating that sufficient substrate was available from bread alone to give maximum pH falls. In general, the degree to which a carbohydrate substrate (a) stimulates the saliva flow, (b) elevates the salivary sugar level, and (c) is retained on tooth surfaces seemed to determine its effect on plaque pH values.

112. THE PERMEABILITY OF MICROBIAL PLAQUES.—*C. Huh, R. Q. Blackwell, and L. S. Fosdick, Northwestern University Dental School, Chicago.* Microbial sludge from incubated saliva-sugar mixtures was vibrated into a previously prepared cell to produce a mat 1 mm. thick and with a surface area of 1 $cm.^2$ The cell above the mat was filled with glucose solution of 0.1, 0.3, 0.5, 0.8 or 1 M concentration. After incubation at 37.5° C. for 5, 15, or 30 minutes, the cell with glucose and mat in place was immersed in a dry ice-acetone mixture until the contents were frozen. Then the cell was broken away and the frozen mat sectioned parallel with the surface into slices 0.1 mm. thick. Each slice was analyzed for glucose and total lactate. It was found that the microbial mat was not freely permeable to glucose, but that such penetration as did occur was in proportion to the concentration of the glucose. The lactate concentration in the mat was also in proportion to the concentration of the glucose. In the deeper slices only the concentrated glucose produced lactate formation on 5 minutes' incubation. With this incubation only very little lactate formed at the open surface. After longer incubation, all glucose concentrations led to lactate formation at the surface and in the deeper layers.

113. REDUCED ORAL TEMPERATURES AND ACID PRODUCTION RATES WITHIN DENTAL PLAQUES.—*Robert J. Adams and William R. Stanmeyer, U. S. Naval Medical Research Laboratory, New London, Conn.* The effect of the reduction of oral temperatures was observed on bacterial reproduction and the acid production rate within the dental plaque. Temperature is an important factor influencing bacterial activity and reproduction. A pH determination on 176 plaques after fasting for a minimum of 2 hours and a pH determination 15 minutes after a 20 per cent glucose rinse were recorded at room temperature. The subjects then worked outside for a minimum of 70 minutes at temperatures ranging from –33.0° C. to –49.4° C., at humidities approaching 5 per cent, During this period the subjects became mouth-breathers. A second 20 per cent glucose rinse was given and the subjects remained working outside for 15 minutes. They were then returned to room temperature and plaque pH determinations were recorded. At room temperature, the hydrogen ion concentration of the 176 plaques showed a significant drop following the 20 per cent glucose rinse. Ninety-six per cent of the plaques did not show a drop in pH following the 20 per cent glucose rinse at these bitter temperatures.

Tooth surface temperatures during the experimental period showed a drop from +30.0° C. to +1.7° C. A marked decrease in oral lactobacillus counts occurred during exposure to the cold.

114. Hydrolytic Enzyme Activity of Dental Plaque.—*Howard H. Chauncey, Tufts University School of Dental Medicine, Boston.* Acid phosphatase, alkaline phosphatase, nonspecific esterase, β-D-galactosidase and β-D-glucosidase levels of plaque were measured. Enzyme activity was expressed as the amount of substrate (microgram) hydrolyzed per unit (milligram) of plaque nitrogen. Material collected from the buccal surfaces of teeth 3, 4, 5, and 6 of all quadrants was used for analysis. Plaque composition was determined after 1, 3, and 5 days' growth. Nitrogen and enzyme contents were observed to increase with increased retention time. Specimens from the different quandrants were analyzed and compared. The lower right quandrant was found to have the heaviest plaque growth but, paradoxically, was lowest in enzyme content. No difference was observed among the other three quadrants. Phi coefficient analysis indicated that there was no correlation between the sediment volume of whole saliva and the rate of plaque formation. However, a positive correlation was observed between plaque enzyme levels and their counterparts in whole saliva.

115. Effect of Saliva Restriction on Dental Plaques in Caries-Immune Individuals.—*H. R. Englander, M. A. Mazzarella, Dental Research Facility, Great Lakes, and L. S. Fosdick, Northwestern University Dental School, Chicago.* Previous studies have shown that the enzyme systems in the dentobacterial plaques on human tooth surfaces are capable of producing lactic acid in large quantities from the residual fermentable carbohydrates of dietary origin. Furthermore, the plaque pH of caries-rampant individuals is unable to return from a low level within 20 minutes whenever saliva is not accessible to the dental plaque. This study attempted to determine the effect of saliva on the pH and lactate concentration of dental plaque from caries-immune persons after a 50 per cent sucrose mouth spray. Plaque material from comparable locations on the upper molars was removed at frequent intervals during a 20-minute period following the application of a sugar solution. The pH and lactate content of the plaques taken in the presence of saliva were compared with those taken in the absence of saliva, and an acid production usually not seen in the plaque material of caries-immune persons appeared whenever the saliva was restricted. It was concluded that while saliva played an important role in acid neutralization, the plaque substance of caries-immune individuals possessed such capacity for buffering that this acid was neutralized almost immediately, instead of remaining at a dangerously low level as in the caries-rampant individuals.

116. Calculus and the Carious Mouth.—*Thomas R. Dirksen, Marquerite F. Little, and H. Scott Wiley, Eastman Dental Dispensary, Rochester.* Two aspects of the dental history of calculus producers, age and caries incidence were studied. In the course of an examination of 790 patients in a hygienist prophylaxis clinic, approximately 216 patients with demonstrable calculus were found. Measurable samples of the calculus were secured from 90 per cent of the calculus patients and identification was confirmed in vitro through the use of soft x-rays. Through examination of the teeth, radiographs, and past dental history, patients were grouped according to caries experience. Preliminary studies were reported on the pH response to sugar in the lesions of teeth with and without calculus. The number of patients with calculus was fairly constant for all age groups. Of the patients with calculus, an approximately equal number (15 to 20 per cent) in both age groups had excessive

deposits. Active caries was found in 70 per cent of the patients with calculus. In the under 30-year age group, 85 per cent had active caries and 90 per cent had open lesions. In the over 30-year age group, at least 50 per cent had active caries and 80 per cent had open carious lesions. The lesions in teeth on which calculus had deposited gave a pH response to glucose similar to that given by the lesions in teeth without calculus.

117. THE EFFECT OF A DENTAL PROPHYLAXIS ON DENTAL CARIES.—*Ralph Lobene, The University of Rochester, School of Medicine and Dentistry.* While the primary purpose of this study was to determine the anti-caries activity of two experimental dentifrices, it was also possible to show the effect of a dental prophylaxis on dental caries. The experimental dentifrices showed no significant reduction in dental caries. A difference, however, was found in the incidence of new caries and the rate of progressive caries between the cleaned and the uncleaned side of the mouth. The subjects were institutionalized teenage boys, drinking fluoridated water. Each subject was examined with mouth mirror, explorer, and bite-wing x-rays. The caries were scored using the methods reported by Shourie, Hein, and associates in *A Dental Survey of Puerto Rican Children.* Following examination, either the right or the left side of the mouth received a dental prophylaxis. Selection of right or left was effectively random. The total number of surfaces scored in the cleaned and the uncleaned side of the mouth was identical at the initial examination. The average number of surfaces of new caries per 100 surfaces that showed no caries at the beginning of the study on the cleaned side was 3.26; on the uncleaned side it was 3.54. The average number of surfaces of progressive caries per 10 surfaces at the beginning of the study was 1.92 for the cleaned side and 2.31 for the uncleaned side. The difference between the two sides in the rate of new caries was about 1.5 times its standard error and not statistically significant at the usual significance level. The difference in the rate of progressive caries, however, was about 2.5 times its standard error and significant at the 2 per cent level. On this basis, it seemed reasonable to conclude that prophylactic cleaning reduced caries activity over a 6-month period.

118. EXPLORATORY RESEARCH IN TOOTHBRUSHING INSTRUCTION FOR PRIMARY SCHOOLS.—*Josse E. de Wever, Dental Medicine Laboratory of the Colgate-Palmolive Company in cooperation with Rutgers University, New Brunswick, N. J., and David F. Striffler, Division of Dental Health, New Mexico Department of Public Health, Santa Fe, N. M.* The impact of three different methods of instruction in a single 20-minute session was evaluated by comparing toothbrushing before and after instruction of 340 children in four elementary schools. In one school the instructor demonstrated on a model the method advocated by the American Dental Association. In the second school, each pupil received an individual brush to mimic in his own mouth the demonstration given on the model. In the third school, the same method as in the second was used, but the instructor added a chart illustrating the 6 buccal, 4 occlusal, and 6 lingual areas. The control group in the fourth school received no instruction. One dental hygienist instructed each first and second grade classroom in all three experimental groups. Recording was done before and after instruction. The observer watched the reflection of the child's movements in a mirror and recorded the brushing method and which of the 16 areas the child attempted to clean. The results were: (1) the use of individual brushes increased the impact of instruction by 53 per cent, (2) the use of a second demonstration tool did not help the instruction, and (3) one 20-minute session of instruction with demonstration on a model did not significantly change toothbrushing practices.

119. The DMFS and RID Indices for Longitudinal Assessment of Dental Caries.—*Donald R. Porter, University of Oregon Dental School, Portland, and John A. Dudman, Reed College, Portland.* The relative increment of decay (RID), an index designed for longitudinal assessment of dental caries, was compared to the DMF surface index commonly used in clinical tests. The records of caries progress for a 6-month interval were selected for 40 children with permanent dentitions only and 67 children with mixed dentitions from records of a 3-year longitudinal study of dental caries progress. The records were selected at random on the basis of the type of dentition present for a 6-month interval; that is, all permanent, or permanent and primary teeth. A correlation coefficient of 0.93 was shown to exist between the change in DMF surfaces and the RID for a 6-month period for the 40 children who had only permanent teeth. For the same 40 children, a correlation of 0.97 was shown between the RID and the ratio of the increase of DMF surfaces to the number of available surfaces. The 67 children with mixed dentition, however, showed an appreciably lower correlation of 0.70 between DMF surface change and the RID for a period of the same duration. Information regarding the status of primary tooth surfaces, frequently omitted in clinical tests for dental caries increments of elementary school children, lessens the confidence in such studies. The RID, which includes all of the units affected and allows for all possible changes occurring in a time interval, permitted comparisons of increments of dental caries in children with different dentitions with a confidence that exists for increments in children with permanent dentitions.

XIV. Anatomy and Periodontal Studies. March 21, Forenoon, Section C.

120. Configuration of the Palate.—*John H. Barr and Poul Grøn, Tufts University School of Dental Medicine, Boston.* Technics for measurement of palate dimensions, developed in a previous study of the optimum locations for placement of x-ray films, were extended to the determination of mean values for dimensions of the palate over its entire area mesial to the third molar tooth. One hundred and thirty-three reference points were devised in workable relationships to available landmarks. In the horizontal plane, those selected were related to the midline of the palate, to a transverse line passing over the labial surfaces of the central incisors, and to the contact points of the maxillary teeth. In the vertical plane, measurements at the reference points were based on a plane related to the tips of the mesiolingual cusps of the first molar teeth. Mean values for 100 adult males and 50 adult females were applied (1) to the production of scale drawings of the palate area carrying contour lines representing 2 mm. increments of height, and (2) to the preparation of casts for use as 3-dimensional representations of the configuration of the palate.

121. A Study of Vertical Dimension.—*Ralph C. Appleby, College of Dentistry, State University of Iowa, Iowa City.* This research was conducted for the purpose of better understanding and analyzing several different methods for determining the physiologic rest position, amount of free-way space, and physical contact position of young adults. This study was conducted by making a series of ten tests and measurements on 140 young female adults with a normal complement of teeth and good muscle control of the masticatory apparatus. The patient was seated in the dental chair, head position upright, yet relaxed, and body position relaxed and unstrained. Two tiny dots were made on the patient's face by means of a fountain pen, one placed just below the base of the nose (subnasion) and one on the point of the chin (gnathion). The patient

was given specific instructions prior to each test for the sake of clarity and uniformity. A measurement of the vertical distance between the two marks was made for each of the ten tests, using a millimeter ruler. The end-of-swallowing test proved positive on 57.15 per cent of our subjects. The range of free-way space measured 1 to 6 mm., and the average free-way space 2.23 mm. The halfway point in swallowing proved positive for the physical contact position on 66.55 per cent of the subjects.

122. IN VITRO CALCULUS FORMATION.—*Frithjof von der Fehr, Forsyth Dental Infirmary, Boston, and Finn Brudevold, Eastman Dental Dispensary, Rochester.* A reproducible quantitative method for in vitro production of calculus was developed which permits independent study of plaque and mineral deposition. Plaque was formed on small standard-sized discs of filter paper, impregnated with plastic, by alternate exposure to saliva and air at 37° C. for 2 to 3 hours. Subsequent immersion of the discs in a calcifying solution for 20 hours caused mineralization. Layers of calculus with a characteristic histologic structure were formed when these procedures were repeated several times. Ca and P analysis, as well as weight increments, showed that mineral deposition increased linearly with time over periods ranging from 2 days to 3 weeks. The Ca:P ratio of the mineral was approximately 2.0 on a weight basis, suggesting hydroxylapatite.

123. ACTINOMYCES AND CALCULUS FORMATION.—*William H. Bowen and Marion N. Gilmour, Eastman Dental Dispensary, Rochester.* As judged by colonial and cellular morphology, a high proportion of Actinomyces colonies were found in every calculus sample cultured from 50 human subjects. Twenty-three strains were isolated for pure culture studies from both human and dog calculus. Pumiced, autoclaved teeth were incubated with pure cultures of (a) Actinomyces, (b) Leptotrichia-like strains, and (c) mixtures of both types of microorganisms in thioglycollate BHI medium which, after several weeks, was fortified with a calcifying solution. Controls were teeth in uninoculated tubes. After 5 to 6 weeks, all teeth incubated with the bacteria exhibited a large calculus-like deposit over the entire root area or on crowns where brown stain had been incompletely removed. No deposit formed on the control teeth. Stains of decalcified and undecalcified sections showed the organisms distributed in clumps throughout the calcified material. All of the Actinomyces strains possessed an acid phosphatase with optimum activity on p-nitro-phenyl phosphate at pH 3.5 to 5.0. In the pH range 7.2 to 8.0, intact cells incubated with cannulated parotid saliva caused a slight but consistent increase in the salivary inorganic phosphate level. All strains required carbon dioxide for growth, and flourished in atmospheres containing up to 40 per cent of the gas. Both properties could result in an enhanced rate of salt deposition.

124. EFFECT OF CERTAIN ENZYMES ON CALCULUS DEPOSITION.—*S. Wah Leung, and Frank J. Draus, University of Pittsburgh School of Dentistry, Pittsburgh.* The effects of several enzymes on dental calculus deposition were tested under the in vitro calculus-producing system previously described. The substrate from which the calculus was produced consisted of a 0.1 per cent solution of a bovine submaxillary gland extract in phosphate buffer at pH 7.5, fortified with $CaCl_2$, and bubbled through with CO_2 until a pH of 6.8 was reached. The enzymes were added to the test solution to a level of 0.05 per cent. The teeth were dipped into the test solutions at a cycle of once every minute. The solutions were changed daily. At the end of 4 weeks, the deposits which had formed on the teeth were sealed, dried, weighed, and analyzed for Ca, P, and CO_2. The results showed that most of the enzymes reduced the amount of deposit formed, as compared to the control system which contained

no enzyme. The percentage reduction was as high as 60 per cent with some of the enzymes. The polysaccharides seemed to be more effective than the proteolytic enzymes. The degree of reduction was roughly correlated with the ability of the enzyme to lower the viscosity of the bovine extract solution. Some of these enzymes were effective even when they were not added to the test solution, but were used merely to rinse the teeth for 2-minute periods, twice a day, during the time the teeth were subjected to the action of the Ca-fortified bovine extract solution. When used in this fashion, however, a higher concentration of the enzyme was required.

125. Esterase Activity Associated With Formation of Deposits on Teeth.—*Paul N. Baer and Marvin S. Burstone, National Institute of Dental Research, U. S. Department of Health, Education and Welfare, Bethesda.* Mylar strips, 6.5 μ thick, were fastened to the lingual surfaces of the lower incisor teeth of patients for periods of 3 to 30 days. The patients were between the ages of 25 and 35. Over 50 strips were used. Upon removal, the strips were incubated to reveal the presence of esterase activity using histochemical technics. The substrates used were acetates of complex naphthols (3-hydroxy-2-naphtho-o-toluidide, and 3-hydroxy-2-methyl-2-naphth-p-anisidide). They were used in a simultaneous azo-dye procedure with Garnet GBS salt (diazotized o-aminoazotoluene) at neutral pH. One-hour incubation periods were used. A number of potential esterase inhibitors were interposed during the reaction period. These consisted of arsenic, fluoride, cadmium, zinc, eserine, Zephiran (alkyl-dimethyl-benzyl-ammonium-chloride), DFP, and Phemerol (benzethonium chloride). Representative sections were also pre-incubated in hot water (90° C.). Deposits exhibited remarkable adherence to Mylar, and masses of bacteria and epithelial cells were well preserved. These structures revealed strong esterase activity. Both cocci and fusiform types were active, with the former showing the highest activity. Polymorphonuclear leukocytes also revealed consistently high activity. Degenerating leukocytes, however, were inactive. Hot water, Zephiran, and eserine produced distinct inhibition, while the effect of the remaining inhibitors was not marked.

126. Four-Week-Old Calculus.—*Stanley P. Hazen and Helmut A. Zander, Eastman Dental Dispensary, Rochester.* To study calculus formation, 29 Mylar strips were placed on the lingual surface of the lower anterior teeth of 11 patients, immediately after cleaning the teeth. Four weeks later the strips were removed and processed for celloidin embedding, sectioned, and stained by various methods. All specimens, with the exception of 4, showed evidence of calcification. Calcification occurred immediately next to the strip, in the center of the mass and occasionally on the surface of the mass. The extent of calcification varied with each tooth and each individual. After calcification had reached a certain stage, bacteria and other cellular material could no longer be demonstrated in such an area. The process of calcification seemed to occur in a regular pattern in a pre-formed matrix. Every specimen showed the presence of microorganisms. They were predominantly gram-positive filamentous forms. The organisms could be found on the strip and throughout the material; however, their number and arrangement varied from specimen to specimen. Metachromatic granules were prominent in the organisms when stained with a stain for diphtheroids. Cells in various degrees of decomposition could be found. They resembled epithelial cells, leukocytes, and lymphocytes. The type of calculus attachment seemed to vary with the smoothness of the strip. The smoother the strip, the more often the appearance of a cuticular structure, while with a rough surface, microorganisms seemed to be directly attached to the strip.

127. HISTOLOGIC STUDIES OF HUMAN PERIODONTAL STRUCTURES UTILIZING FLUORESCENCE MICROSCOPY.—*Alvin C. Hileman, College of Physicians and Surgeons, San Francisco.* The purpose of this investigation was to determine the usefulness of fluorescence microscopy in histopathologic studies of human periodontal structures. Upper anterior teeth with a wedge of attached gingiva and alveolar bone were removed from patients having extractions for dentures. The specimens were fixed in 10 per cent formalin, decalcified in 5 per cent nitric acid, and prepared for sectioning in paraffin. Two hundred sections were prepared from five specimens. Several sections were prepared for primary fluorescence observation without staining. Tissue differentiation was found to be unsatisfactory with this technic. A large number of sections were stained with a series of anodic, cathodic, and electroneutral fluorochromes at varying pH values for secondary fluorescence observation. Acridine orange and berberine sulfate were superior single stains. One combination, Congo red followed by berberine sulfate, revealed interesting tissue differentiation patterns. Secondary fluorescence color values of the various tissues could be regulated by changing the pH of the staining solutions. Acridine orange was the most sensitive fluorochrome in this respect. This was apparently related to the isoelectric point of the tissue proteins. An outstanding feature of the secondary fluorescence studies was the clarity of the epithelial attachment cuticular structures. Comparative studies were made by staining some sections with hematoxylin and eosin and Mallory's triple connective tissue stain for conventional visible light microscopic observation. Cellular morphology of the connective tissues was more clearly defined using visible light microscopy. This was partly due to increased intensity of radiation available. A greater intensity of ultraviolet radiation would undoubtedly make fluorescence microscopic technics more useful for studies of this nature.

128. CHARACTERIZATION OF HUMAN GINGIVAL EPITHELIAL INTERCELLULAR CEMENTING SUBSTANCE.—*John C. Thonard and Henry W. Scherp, University of Rochester, Rochester.* To validate any hypothesis concerning the role of various bacterial enzymes in the initiation of gingivitis, the specific substrates in the gingivae must be identified; very little is known about the localization of these components or about the nature of the intercellular cementing substances of epithelium. Fresh gingival tissue, normal and periodontitic, was sectioned from conventional paraffin infiltration and freeze-dried specimens and stained by the Hales and periodic-acid-Schiff (PAS) technics and with toluidine blue at a pH of 6.0 and 2.5. Additional serial sections were treated before staining with hyaluronidase (animal and bacterial), chondroitinase, and filtrates from mixed oral bacterial cultures, with parallel heat inactivated enzyme control sections. It was shown that gingival epithelium contained (1) Hale's positive intercellular material, which was not removed entirely in vitro by hyaluronidase or chondroitinase or mixed culture filtrates; (2) PAS-positive intercellular material, not removable with hyaluronidase and chondroitinase and only partly removed by amylase; and (3) material metachromatic at pH 6.0 and 2.5, not entirely removed in vitro by the enzyme preparations employed. The crevicular epithelium of gingivitic tissue showed vacuolization of the intercellular spaces with much loss of staining. The results indicate that (1) epithelium contains acid mucopolysaccharides; (2) the nonremovable moiety of the intercellular cementing substance could be chondroitin sulphate B or an unknown component giving characteristic mucopolysaccharide staining reactions, (3) in vivo there is disaggregation of the intercellular mucopolysaccharides, presumably due to unidentified bacterial "enzymes"; and (4) depolymerization of the intercellular substance of the epithelium is possible as the first step in the pathogenesis of periodontal disease.

129. Root-Surface Resorption of Periodontally Diseased Teeth.—*Brian L. C. Harvey and Helmut A. Zander, Eastman Dental Dispensary, Rochester.* The relationship of cementum thickness with age was studied on the roots of teeth with healthy and diseased periodontal structures by Zander and Hürzeler. The average thickness of the cementum was remarkably less on periodontally diseased teeth and on teeth obtained from India than on those that could be called "healthy." From these studies arose the question of whether the differences in cementum thickness observed could have been brought about by a decrease in the rate of cementum apposition, or whether the cementum apposition remained the same but resorption had occurred at an excessive rate. Therefore, the number and size of resorption areas were studied on the same material which Zander and Hürzeler used for their studies. More resorption was found on the roots of the teeth from the periodontally diseased and "the India group" than on the "healthy" tooth group.

130. Effect of Inflammation on Mitosis in Gingiva at Two Ages.—*A. S. Marwah, University of Illinois College of Dentistry, Chicago.* Mitotic rate was studied in gingival biopsies obtained from men of 25 to 34 and 50 to 78 years of age. The individuals included in this study had clinically normal gingivae. Five sections, 6 μ thick and 30 μ apart, were studied from each specimen. Separate counts were made of the crest and oral surface region of the free gingiva in inflamed and uninflamed areas. The total cell population ranged from 6,000 to 26,000 cells per region per specimen. The number of cells in mitosis in each of the two subregions of the free gingiva was counted. The total number of epithelial cells containing these mitoses was estimated from a planimetric measurement of the surface area in drawings of projected slides and from counts of cells in measured sample areas. In inflamed regions, type, location, and density of inflammatory cells were evaluated. In the clinically and histologically uninflamed gingiva, the mitotic index in both age groups was slightly higher in the crest region than at the oral surface of the free gingiva. In the young group, it averaged 0.55 at the crest and 0.49 at the oral surface. In the old group, the average was 0.84 at the crest and 0.76 at the oral surface. Inflammation caused a rise to more than double the normal in the average mitotic frequency in each region at each age level. Thus, in the crest region, the mitotic index rose from 0.55 to 1.51 for the young group and 0.84 to 1.92 for the old-age group. In the oral surface of the gingiva, the mitotic index rose from 0.49 to 1.08 for the young group. It was less marked for the old-age group, but rose from 0.76 to 1.10. Although the effect of inflammation was a statistically and numerically significant increase in the group average in all 4 subgroups, the effect was variable in individual specimens, some cases showing no effect at all, whereas others showed greatly accelerated cell division.

131. Some Age Changes in the Periodontium of Albino Mice.—*Neville D. Gilmore and Irving Glickman, Tufts University School of Dental Medicine, Boston.* Age changes in the periodontium of adult female albino mice, R.A.P. strain, were studied in two age groups; from 1 to 7 months (37 animals), and from 12 to 16 months (13 animals), by examining buccolingual sections of functioning mandibular first molars. Growth changes occurred in the alveolar bone and cementum and were apparently related to movement of the teeth in an occlusobuccal direction. The bone changes consisted of apposition and resorption in relation to the periodontal membrane, the crests of the buccal and lingual plates, and the buccal and lingual periosteum. Cemental changes consisted of apposition, mainly in relation to the apical third of the tooth. The above changes occurred intermittently, and were more prominent in the younger group. In the older group, the buccal and lingual plates showed apposition

and resorption lines which indicated previous bone activity consistent with the growth pattern. At all ages there were areas of compression and tension in the periodental membrane and associated resorption and formation of adjacent alveolar bone, apparently related to intermittent and changing buccolingual functional forces, and independent of the over-all pattern identified with the occlusobuccal movement of the tooth. At the age of 1 month, the epithelial attachment was on the enamel; at 16 months, it was on the cementum, often exposing some root surface. Gingival inflammation associated with food particles and/or calculus was seen more often and was more severe in older animals. Resorption of the underlying alveolar crest was also seen.

XV. Experimental Carcinogenesis and Keratosis. March 21, Afternoon, Section A.

132. The Effects of Tween 80 and Prednisilone Acetate on Chemical Carcinogenesis in the Hamster Cheek Pouch.—*W. R. Sabes, A. P. Chaudhry, and R. J. Gorlin, School of Dentistry, University of Minnesota, Minneapolis.* Sixty-seven golden Syrian male hamsters, approximately 40 days old, were divided into four groups of 20, 20, 19, and 7 animals, respectively. The animals in Group I were treated by painting the left cheeck pouch with pure Tween 80 (polyoxyethylene sorbitan mono-oleate). After drying for 1 minute, the previously painted area was swabbed with 2 drops of 0.5 per cent 7:12 dimethylbenz (α) anthracene in liquid petrolatum. The animals of Group II were similarly treated by swabbing the left bucal pouch with 0.025 per cent prednisilone acetate (Sterane) followed by the application of the carcinogen. Group III was painted with only the carcinogen. Group IV was used as a control and was painted with Tween 80 alone. No control group for liquid petrolatum was employed since its noncarcinogenic properties have been repeatedly established in this laboratory. The paintings were repeated every third day for a total of 27 applications over a period of 80 days. The latent period in Group II (Sterane-treated) animals was 42 days compared with Groups I and III, in which it was 60 and 63 days, respectively. Furthermore, at any stated time interval, the tumor incidence was higher in the former group of animals than in the latter group. This difference was statistically significant between 60 and 81 days. Microscopically, the incidence of invasive squamous cell carcinoma was found to be higher in the Sterane-treated group than in the Tween 80-treated group ($P = <0.02$) at the end of the experiment. No such difference was noted in the Sterane-treated and the carcinogen-treated groups.

133. Experimental Carcinogenesis in Submaxillary Glands of Hamsters.—*A. P. Chaudhry, and R. J. Gorlin, School of Dentistry, University of Minnesota, Minneapolis.*—This study has been conducted with three objectives. First, to investigate the histopathologic changes at various intervals of time in the submaxillary gland of the hamsters following a single injection of a hydrocarbon carcinogen. For this experiment, 50 golden Syrian male hamsters, 3 months of age, were used and were divided into six groups. The animals were singly housed in a room maintained at 70° ± 2° F. and a lighting regimen of 6 A.M. to 6 P.M. and darkness from 6 P.M. to 6 A.M. Under intraperitoneal Nembutal anesthesia, the ventral surface of the neck was opened and the submandibular glands exposed. The right gland was injected with 0.05 c.c. of 0.5 per cent 7:12 dimethyl benzanthracene dissolved in liquid petrolatum. The left gland was used as control and injected with the same amount of solvent. The animals from the first to the sixth groups were sacrificed at intervals of 1, 4, 8, 10,

14, and 18 weeks, respectively, and the glands studied for microscopic changes. Coagulation necrosis, metaplasia of ducts, and scarring followed by malignant changes of connective tissues were evident. At the fourteenth week, 33 per cent of the animals developed fibrosarcoma of various gradation. At the end of 18 weeks, the incidence was nearly 45 per cent. The second objective was to study the effects of different concentrations of a single injection of the carcinogen on the number of tumors induced. All other factors were kept constant except that 0.05 c.c. of 0.1, 0.25 per cent, and 1 per cent of dimethyl benzanthracene was injected into three groups of the animals, respectively. At the end of eighteenth week, a statistically significant difference in the incidence of tumors was apparent. Third, study of the effect of age on the experimental induction of tumors by the chemical carcinogen was carried out. Species, sex, environmental conditions, amount and concentration of the carcinogen were all kept constant except for age. No statistical difference in the incidence of tumors was apparent in 4 groups of animals of 1, 3, 6, and 9 months, respectively.

134. Effects of Vitamin A Deficiency on Experimental Carcinogenesis in Submaxillary Glands of Hamsters.—*A. P. Chaudhry, Leon Singer, and R. J. Gorlin, University of Minnesota, Minneapolis.* Eighty-two golden Syrian male hamsters, 8 weeks of age, were divided into three groups of 50, 15, and 17 animals, respectively. The animals were singly caged in a room maintained at a temperature of 70° ± 2° F. with a lighting regimen from 6 A.M. to 6 P.M. and darkness from 6 P.M. to 6 A.M. Under intraperitoneal Nembutal anesthesia, the submaxillary glands were exposed. The right gland was injected with 0.05 c.c. of 0.5 per cent 7:12 dimethylbenz (α) anthracene. The left gland was used as a control and injected with the solvent liquid petrolatum. Three days postoperatively, the animals in Group I were put on a vitamin A-deficient diet and were fed ad libitum. The diet was specially prepared in bread form and was chemically analyzed for vitamin A level. The second group of 15 animals was given the same diet but supplemented with 1,000 I.U. of vitamin A per week. This group was pair-fed with the animals of Group I. The third group of 17 animals was given the same bread supplemented with 1,000 I.U. of vitamin A per week but this group was fed ad libitum. At the end of 18 weeks the animals were sacrificed and the number of tumors recorded. No significant statistical difference in the incidence of tumor was found between Groups II and III, and, they were therefore, combined for the purpose of statistical analysis. The incidence of tumors in Group I was 25.5 per cent, and 16 per cent in the combined Groups II and III. This difference was not statistically significant ($P = > 32$ per cent). No direct relationship was found between plasma vitamin A levels and liver storage. The serum vitamin A level was maintained at near normal when the liver stores were almost depleted in animals fed on the vitamin A-deficient diet.

135. The Transplantable BW1081 Mouse Salivary Gland Tumor and X-Radiation.—*James O. Beck, Jr., Indiana University, Indianapolis.* The effects of x-radiation on the growth pattern of the BW1081 mouse salivary gland tumor and on the host responses to the transplanted tumor were studied. The groups of mice exposed to x-rays for this study received 250 or 400 r total body radiation as follows: (1) x-radiation of stock tumor mice with tumors of varying ages and sizes, (2) x-radiation of the tumor prior to its being transplanted to mice which had not been exposed to x-rays, and (3) x-radiation of mice prior to their receiving tumor transplants which had not been exposed to x-rays. Under these experimental conditions, the BW1081

tumor was not destroyed by the amount of radiation used; however, the tumor growth rate was retarded. Marked reductions also were observed in the usual manifestations of the myeloid leukemoid response of the host to the transplanted tumor, as determined by peripheral white blood cell counts and histologic study of the liver, spleen, and bone marrow.

136. EXPERIMENTAL OSTEOGENIC SARCOMA OF THE JAWS OF MICE.—*Barnet M. Levy, University of Texas Dental Branch, Houston.* The fundus of the mandible of twenty Swiss mice was surgically exposed. The fundic bone was removed, and approximately 0.05 c.c. of 1 per cent methylcholanthrene in olive oil was placed in the bone cavity and covered with 4 per cent celloidin. Animals were sacrificed at various time intervals, and the heads were prepared for histologic study. The bone wound was completely healed in 2 months, and an abnormal type of osteodentin filled the periodontal area. Odontoma-like masses were seen. Later there was evidences of malignant change in the endosteum. By 9 months following the implantation of the carcinogen, tumors with the histologic pattern of osteogenic sarcoma were seen. These were large tumors which invaded the buccal plate of bone and masseter muscle, as well as the lingual plate of bone and the floor of the mouth.

137. SARCOMA INDUCTION BY IMPLANTATION OF DENTAL MATERIALS IN RATS.—*David F. Mitchell, Govind B. Shankwalker, and Shirley Shazer, Indiana University School of Dentistry, Indianapolis.* Nickel, other metals, and plastics have been shown to induce tumors in rats and other rodents. Since many materials are being implanted for various reasons in the jaws of human beings, it seemed worth while to test for a possible carcinogenic effect of a variety of dental materials. One hundred and twenty young adult Wistar rats were distributed equally according to sex into 12 groups. All animals received 4 subcutaneous implants of small pellets (approximately 2 by 2 mm.) of one of the following: fused porcelain beads, silver wire, zinc oxide and eugenol, "Pulp-dent," silver amalgam, autopolymerizing resin (Kadon), copper, silicate, denture resin, Vitallium (Cr-Co-Ni alloy), gallium (40 per cent)-nickel (60 per cent) alloy, and nickel. Rapid growing, metastasizing, transplantable fibrosarcomas developed around the nickel and nickel-gallium pellets as early as 7 months after implantation. Nine of the 10 animals of the nickel-gallium group developed tumors within the first year. One animal died without tumor. Of the 10 animals in the middle group, 2 developed tumors between 7 and 8 months—1 at 15 months, and 1 at 17 months. Three animals died, and 3 are living without tumor. The tumors were single in each animal except for 1 Ni-Ga animal which developed 2 tumors. No tumor was found in any of the other groups except for one spontaneous, benign breast tumor. There was no apparent sex difference related to tumor induction. The histopathologic appearance of the tumors and metastatic nodes were characteristic for a high degree of malignancy. The rate of growth of the tumors, once established, was great.

138. EFFECT OF TOBACCO SMOKE AND ULTRAVIOLET IRRADIATION ON LIPS AND EARS OF MICE.—*John J. Salley, Medical College of Virginia School of Dentistry, Richmond.* In previous studies, daily applications of controlled volumes of whole tobacco smoke alone have failed to produce any significant changes relative to epithelial neoplasia in mice, even when exposure to tobacco was carried on past one half the average life span of the animal. The present investigation was designed to observe the effect of prolonged application of whole cigarette smoke plus concurrent exposure to ultraviolet irradiation on

the lips and ears of Swiss and CAF_1 strain mice. Groups, consisting of 40 mice each, were treated daily for periods up to 95 weeks as follows: (1) smoke plus ultraviolet, (2) 0.2 per cent benzpyrene plus ultraviolet, (3) ultraviolet alone, and (4) 0.2 per cent benzpyrene alone. Results showed that carcinoma of the ear was induced in 3 animals exposed to smoke plus UV. Lips in this group were negative. Ultraviolet irradiation alone resulted in ear tumors in 5 animals with no significant changes noted on the lips. Benzpyrene-treated mice all presented malignant cutaneous tumors on the ears with a reduced latent period for tumor induction in the group which received daily exposure to UV. The labial mucosa of these animals was negative. There were no significant strain differences. No significant difference in incidence of neoplasia was demonstrated between the smoke plus UV-treated animals, and those receiving UV alone. When UV irradiation was concurrently delivered to ears which were treated with a known carcinogen, there was however, an apparent synergistic effect with reference to latent period.

139. A Histochemical Study of Pathologic Keratosis of the Oral Mucosa.—*Samuel Turesky, Irving Glickman, and Jacqueline Provost, Tufts University School of Dental Medicine, Boston.* Oral lesions (80), diagnosed clinically as leukoplakia, were studied histochemically in an effort to obtain a better understanding of pathologic keratosis as it occurs in human oral mucosa. Serial sections were stained with the periodic-acid Schiff (PAS) technic for glycogen and carbohydrate protein complexes and the Barrnett-Seligman technic for protein-bound sulfhydryl groups and disulfide linkages. Hematoxylin-eosin stained sections provided orientation. All areas of the oral cavity were represented in the biopsy specimens, which presented varied degrees of hyperkeratosis and/or parakeratosis. Hyperkeratosis was characterized by a decrease in sulfhydryls and a pronounced increase in disulfides. Glycogen was reduced in the underlying epithelium. In parakeratosis, there was a concentration of sulfhydryls in the stratum corneum but no increase in disulfides. In many specimens, the stratum corneum presented alternate keratinized and parakeratinized bands which differed in sulfhydryl and disulfide content, suggesting that keratinization could occur as an intermittent process.

140. Experimental Production of "X" Disease (Hyperkeratosis) in Hamsters With Chlorinated Naphthalenes.—*Alvin L. Norris and Stephen F. Dachi, University of Pennsylvania, Philadelphia.* "X" disease and hyperkeratosis are names applied to a disease in cattle which is characterized by hyperkeratotic alteration of the oral mucosa and skin, and degenerative changes of the kidneys and liver. When the condition was produced experimentally by feeding chlorinated naphthalenes, a disturbance of vitamin A metabolism was noted. The purpose of this experiment was to determine if the disease could be produced in hamsters. Albino hamsters, each weighing about 130 grams, were separated randomly (as to sex) into 7 groups of 6 animals. Octachloronaphthalene suspended in carboxymethylcellulose, Tween 20 and water were applied topically 5 times weekly via a 16 gauge needle in the oral pharynx of Groups A, B, C, and D in daily doses of 10.1, 1.0, 0.1, and 0.01 mg., respectively. After 4 weeks, the dosages of Groups C and D were changed to 500 and 100 mg., respectively. Group E received only the vehicle and Group F served as untreated controls. Group G had the suspension applied topically to the right cheek pouch. After 12 weeks, the animals were sacrificed and paraffin sections, prepared from all organs, were stained with hematoxylin and eosin. Liver vitamin A determinations were carried out using the Carr-Price method. No changes were observed in Groups A, B, E, and G. Animals of Group C

revealed striking changes of the incisor teeth characterized by the chalky whiteness and increased translucency. The livers of these animals were pale and mottled and microscopically revealed marked cloudy swelling. Vitamin A levels in these livers averaged 646 units ± 169 as compared to 1,086 units ± 272 in control animals. It was concluded that symptoms compatible with mild X disease were present in these animals.

141. IN VIVO TISSUE CULTURE UTILIZING THE DIFFUSION CHAMBER TECHNIC.—*S. M. Standish, Indiana University School of Dentistry, Indianapolis.* The diffusion chamber technic, as devised by Algire, has been utilized by various investigators to maintain or grow autologous, homologous, or heterologous tissue fragments in sealed capsules of cell-impenetrable cellulose (Millipore) filters implanted intraperitoneally, subcutaneously, intramuscularly, or intravenously. This technic of tissue transplantation was employed in pilot studies to clarify various technical details of the method which have not been previously reported. The diffusion chambers were prepared by cutting cellulose acetate butyrate tubing into thin rings and gluing the filter to either side of the ring after enclosing the tissue fragment. Tissues used included isologous and homologous salivary gland, tongue epithelium and muscle, and trypsinized BW 1081 tumor (malignant myoepithelioma) cells. Various filter types, with porosity grades from 1.2 to 0.25 μ were tested. The effect of increased diffusion distance for tissue fluids was tested by utilizing plastic rings of varying thickness, ranging from 1.0 to 5.0 mm. Various agents were employed to glue the filter to the ring. The effect of storage time on tissue viability was tested and normal saline and No. 199 tissue culture media were used to store tissues prior to implantation. Sterilizing technics, tissue fragment size, and implantation site were likewise tested. Factors deemed most essential for survival of cells implanted in diffusion chambers were minimal diffusion distance, sterile technic, filters with average pore size of 0.4 μ, perfect sealing of chamber, small tissue fragments or slices, and the use of less specialized tissues.

142. DETECTION OF INTRAORAL CARCINOMA IN SITU WITH EXFOLIATIVE CYTOLOGY.—*Sol Silverman, Jr., University of California, San Francisco.* It was found that characteristic malignant squamous cells were, in some cases, shed from mucosal surfaces in the very beginning stages of cancerous change, prior to the conventionally accepted clinical signs. This finding alters previous reports which state that identification of malignant cells by exfoliative cytology in intraoral leukoplakia, prior to ulceration, is of no value. This series of studies further indicates, for the first time, that intraoral exfoliative cytology can be extremely useful in prematurely indentifying exfoliating malignant cells in carcinomas that show no histologic evidence of invasion. It is only at this stage that we can hope to acquire a satisfactory cure rate in a disease with ordinarily a very poor prognosis. This study was concerned with only those lesions where biopsy was not indicated as an immediate diagnostic aid. The lesions were predominately either keratotic mucosal alterations without ulceration and induration, or soft, freely movable ulcerations, less than a centimeter in diameter. Of the 100 patients examined in this series, 7 had cells consistent with malignancy. This evidence prompted immediate excisional biopsies with rather wide margins. There were no false positives, and as evidenced by follow-up studies, no apparent false negatives. Serial histologic sections were necessary in all cases to observe the malignant changes. Six specimens were diagnosed as squamous cell carcinoma in situ, and one showed questionable evidence of very early invasion. The most common cellular morphologic changes were those of pleomorphism, hyperchromatism, and an altered nuclear-cytoplasmic ratio.

XVI. Caries: Human. March 21, Afternoon, Section B.

143. Manometric Studies of Metabolic Factors in Caries-Rampant and Caries-Immune Saliva.—*George H. Green, Dental Research Facility, U. S. Naval Training Center, Great Lakes.* Previous investigations have demonstrated that there is a highly significant difference between the oxidation of certain substrates by the saliva of caries-rampant as compared to that of caries-immune subjects. The saliva of the caries-rampant group exhibited a higher rate of metabolic activity. In the present study, saliva samples from 25 caries-rampant and 25 caries-immune subjects were separated into various components in order to determine if any one part could be responsible for these characteristic metabolic reactions. The endogenous respiration and the oxidation of glucose and lactate by these various fractions were measured using conventional manometric technics. The respiration rates of supernatant fluid from centrifuged whole saliva, pure parotid secretions, and washed salivary sediments were compared to those of whole saliva. In every instance, the metabolic activity recorded for each of these individual components was only a small fraction of that of whole saliva. When the supernatant fluid from each sample was recombined with its own washed sediment, its metabolic activity was fully restored. The addition of caries-immune supernatant to caries-rampant sediment also restored the activity of that sediment to its normal value; however, the addition of its autogenous parotid or of caries-immune parotid secretions depressed its activity to the caries-immune level. The normal activity of caries-immune sediment was restored by the addition of autogenous or caries-rampant parotid secretions, but was stimulated to the caries-rampant level of activity by the addition of caries-rampant supernatant.

144. The Agglutinin Titer of Parotid Saliva.—*Seth W. Gilkerson and Robert B. Wolcott, Dental Research Laboratory, U. S. Naval Training Center, Bainbridge, Md.* The agglutination reaction between parotid saliva and oral lactobacilli was investigated. The saliva samples were collected from caries-free and caries-active individuals by the use of parotid cups influenced by paraffin stimulation. Each sample was concentrated by evaporation to one fifteenth of the original volume. By recording spectrophotometry, each concentrate was analyzed for total protein content. The values ranged from 1.23 to 3.90 per cent. Formalin-attenuated lactobacilli were used for the antigen suspension. The culture of the lactobacilli was isolated on a selective medium from a caries-rampant patient. The tube, serial dilution method was employed for any observable consequence of antigen-antibody combination. Each dilution was checked both macroscopically and microscopically. The highest titer obtained was 1-512, on a sample from a caries-free subject. The lowest titer of 1-2 was found in a concentrate from a caries-active subject. The average titer of 10 caries-free individuals was 1-203; an equal number of caries-active persons gave an average titer of 1-87. It was suggested that this antibody may be important in the acquirement and maintenance of caries immunity.

145. The Isolation of Collagenolytic Oral Bacteria by Enrichment Culture.—*Solon A. Ellison and Paul A. Mashimo, College of Physicians and Surgeons, Columbia University, New York.* Eleven varieties (28 strains) of bacteria were isolated from carious dentine by enrichment culture, employing undecalcified enamel and dentine, as substrates. Only two types, a gram-negative aerobic rod and a gram-positive coccus, were proteolytic. Tests on natural and synthetic substrates indicated collagenase and aminopeptidase production since attack was limited to cattle Achilles' tendon, rat-tail collagen, Azocol, gelatin, and leucyl-glycyl-glycine. Studies using salt, alcohol, and acetone fractionation, and column chromatography on hydroxyapatite showed that

considerable purification could be achieved, particularly by the latter procedure. The two types of enzymatic activity were not separable. These enzymes appeared to be true collagenases but differed in substrate specificity from the only other known bacterial collagenases, those of the Clostridia. The results indicated that although proteolytic bacteria may be selected by enrichment culture with appropriate media, not all the organisms, so isolated, attack common proteins or peptides.

146. YEAST-LACTOBACILLUS-SALIVA INTERRELATIONSHIPS.—*Paul W. Goaz, Thomas E. Wilson, and Hal H. Ramsey, Dental Section, Oklahoma Medical Research Foundation, Oklahoma City.* Early studies of the oral yeast-lactobacillus relationship led to the conclusion that the apparent symbiotic activity of these microorganisms was the result of a complementary effect of the glycolytic enzymes of each. The major thesis of this hypothesis however, was contingent upon the speculation that the hexose phosphates provide a more readily available energy source for the lactobacillus than does glucose. It was demonstrated, both in the presence and absence of a filtrate from a young *Candida albicans* (oral strain) broth culture, that these phosphorylated hexose intermediates of the glycolytic system could not be substituted for glucose in (1) growth initiation, (2) the support of actively metabolizing cultures or (3) the conversion to lactic acid by nongrowing suspension of an oral lactobacillus. Vitamins required for growth or acid production by this lactobacillus were replaced in effective amounts by *C. albicans* in a culture system designed to demonstrate the effect of factors actively secreted by the young yeast cells. The significance of these growth factors, secreted by the yeast and demonstrated in an experimentally deficient medium, is limited, since no enhancement of growth was observed in the deficient medium conditioned by yeast, when it was fortified with autoclaved saliva.

147. DENTAL CARIES REDUCTION BY THE USE OF BACTERIAL ANTAGONISTS.—*Richard R. Rutter, Walter G. Ruefenacht, Cloyd R. Chamberlain, Paul R. Thomassen, Marian Rose, and Charles A. Scrivener, College of Physicians and Surgeons, San Francisco.* The presence of bacterial antagonists in predominant numbers in the dental plaque may provide a protective barrier between the dental enamel and acidogenic organisms found free in the saliva. Early efforts at implanting microorganisms into the mouth resulted in failure. The organisms disappeared within a few minutes after inoculation. Patients were, therefore, inoculated using the following technic: Complete examinations were conducted, using mirror and explorer and bite-wing roentgenograms. The patient rinsed his mouth with a solution of bacitracin and dihydrostreptomycin

Resulting Data	*Experimental Group*	*Control Group*
No. of patients	46	101
Average age	25.6	25.8
Sex distribution		
Male	54%	34%
Female	46%	66%
Average number of months between examinations	16.4	16.4
Average number of teeth at first examination	26.3	24.9
Average number of DMFT-deft at first examination	18.1	18.4
New DMFT-deft—Total	34	150
Average number of new DMFT-deft per subject	0.74	1.48
Patients showing no new DMFT-deft	74%	50%

to reduce the oral bacterial population. The mouth was then rinsed with tap water to remove the excess antibiotic solution. A suspension of viable vegetative and/or spore forms of *Bacillus brevis* (the choice of antagonists) was generously applied to all enamel surfaces. The patient was requested not to brush his teeth for 24 hours. By adapting the organism to thrive in oral conditions and by improving methods of inoculation and recovery, we could expect every inoculated person to retain the organisms for an average of 30 days. In some instances, the organisms remained for more than 7 months. The increment of DMFT-deft in the *B. brevis* group was 50 per cent that of the control group. This difference was significant at the 5 per cent confidence level. A comparison of the percentage of patients with no DMF or def increment was made between the two groups. These percentages were 74 per cent in the *B. brevis* group and 50 per cent in the control group. Using the chi-square test, the difference in this respect was found to be statistically significant at the 1 per cent level.

148. Bacterial Decomposition of Enamel and Dentine In Vitro.—*Arthur N. Bahn and Helmut G. Haberstroh, College of Medicine and Dentistry, University of Illinois, Chicago.* Weight loss, changes in the calcium and organic content of tooth slices, and changes in the hydrogen ion concentration were determined after immersing 140 sterile tooth slices for 30 days in a casein hydrolysate-glucose medium inoculated with *Lactobacillus acidophilus, Streptococcus salivarius,* or *Streptococcus faecalis* var. *liquefaciens,* individually or in combination. Tooth slices approximately 1 mm. thick were prepared from unerupted human third molars and sterilized. The media were renewed every 72 hours. It was found that *L. acidophilus* produced the greatest weight loss and the largest loss of calcium in the tooth slice as measured by oxalate titration, both in pure and combined cultures. *Strep. faecalis* produced only slightly less weight and calcium loss and slightly less acid than *L. acidophilus* both in pure and combined cultures. *Strep. salivarius* was the least active of these three microorganisms. Control slices in uninoculated media showed no weight, calcium, or organic loss. No significant amounts of the total organic material, as measured by the acid permanganate colorimetric analysis, were removed by any of the three microorganisms studied. Total weight loss could be accounted for essentially by calcium loss. These data suggest that microbial decomposition of enamel and dentine in the casein hydrolysate glucose medium was primarily the result of decalcification.

149. Factors Influencing pH Differentials of Glycolyzing Salivary Sediment.—*R. S. Manly, Tufts University School of Dental Medicine, Boston.* The extent of pH drop in thin films of oral microorganisms undergoing glycolysis has sometimes been assumed to be related to the caries susceptibility of the patient from whom saliva was obtained and to be influenced by the thickness or density of the film. The influence of these three variables was studied through their action on pH differentials of thin, glycolyzing films of salivary sediment. Investigations were performed at three levels of thickness (0.2, 0.4, and 0.6 mm.), and three levels of density, ranging from 5 to 12 per cent dry weight. Four to eight determinations were performed at each level of thickness and density. The pH differentials were greater with high density and/or thickness, but thickness was generally more influential than density change. The resistance of films to an inhibitor, 0.01 per cent iodoacetate, was increased for thick films, but not for dense films. Preliminary studies were made of the influence of caries susceptibility of persons from whom the saliva was obtained. Salivas from 10 caries-susceptible and 10 low-caries persons were compared, using both high and low density sediment. The average pH differentials were the same for both types of saliva, but the resistance to iodoacetate inhibition

differed by 10 per cent. This difference held both for high and low density sediments. These findings suggest that pH differentials obtained from salivary sediment have questionable value as indices of caries susceptibility of the individuals or groups and that the thickness of the sediment is more important for determining the pH differential than is the solid content.

150. PROTECTIVE COATINGS ON ENAMEL SURFACES.—*Robert C. Caldwell, University of Alabama Medical Center, Birmingham.* It has been reported that certain foodstuffs stick tenaciously to enamel surfaces. Consumption of such foodstuffs has often been associated with high dental caries activity. A possible method for reducing the retention of sticky foods on teeth is to coat the tooth surface so that the force of adhesion is minimized. Organo-silicon, organo-phosphorous and organo-titanium compounds were investigated in this study in the hope that a suitable coating could be developed as a caries-preventive agent. Coatings were evaluated according to the reduction in the adhesion of foods, resistance to acids, stability and durability in water and saliva, and by a simple test for water repellency of the surface. It appeared that polymeric materials such as Dow Corning 200 Fluid (a dimethylpolysiloxane), or Union Carbide Corporation's R-23 (a silicone resin) have the desired properties of low toxicity, good esthetics, durability, and stability. Attempts to use such compounds directly on the enamel surface had only a transitory effect. However, by chemically pretreating the tooth surface with a primer such as tetrabutylorthotitanate, the coating could then be applied and would hold to the tooth in a satisfactory manner. Alkoxysilanes applied as aqueous suspensions freshly polymerized, or as monomers which are polymerized on the teeth, formed coatings which had a high degree of water repellency initially, but were less permanent than the coatings formed by polymeric resins.

151. ALTERATIONS IN SALIVARY LACTOBACILLI COUNTS FOLLOWING ESSENTIAL DENTAL PROCEDURES IN CHILDREN.—*W. J. Carter, J. E. Wells, and M. E. Greer, The University of Kansas City, School of Dentistry, Kansas City, Mo.* The effects of a restricted carbohydrate diet on salivary lactobacilli are well known. Recently Shklair (J. A. D. A. 53: 155, 1956) and Kesel (J. D. Res. 37: 50, 1958 [Abst.]) demonstrated a precipitous drop in oral lactobacilli following operative replacement of carious lesions in young adults with silver amalgam and silicious cements. This reduction in lactobacilli was maintained for periods varying from 10 to 18 months. The experimental methods of Shklair and Kesel were duplicated on 38 children, ranging in age from 6 to 12 years. Saliva samples were obtained before operative procedures, following completion of all essential dental procedures, and at 1- and 2-year intervals. The organisms were cultured and colonies enumerated from Rogosa media. It was demonstrated that the salivary lactobacilli counts were reduced from over 10,000 col./mm. to less than 2,000 col./mm. in 81 per cent of the cases. One year later, 58 per cent of the patients had counts less than 2,000 col./mm. Prior to operative procedures, 39 per cent had negative counts, and upon completion of dental care, 59 per cent were negative. In the second year, only 32 per cent had negative counts. No association was found between total lactobacilli counts and past caries indices.

152. EFFECTS OF STORAGE UPON THE BUFFERING ABILITY OF STIMULATED SALIVA.—*C. K. Claycomb, D. C. Gatewood, and M. L. Snyder, University of Oregon Dental School, Portland.* Evaluation of acid buffering ability of stimulated saliva was part of a long-term periodic investigation of laboratory tests as predictors of caries activity. Since the children under study were located 50 miles from the laboratory, it appeared necessary to establish the limits and

conditions for storage of specimens prior to analysis. These were determined by means of data obtained with specimens of saliva from 80 dental students. Five-minute paraffin-stimulated specimens were collected. After measuring the total volume, each of the samples was divided to give four 2.0 ml. aliquots, one of which was immediately brought with 0.002N HCl to pH 6.0 and then to pH 5.0, using a Beckman automatic titrator. The remaining aliquots were stored under varying conditions and intervals of time before thawing and titration. The milliequivalents of acid used per 2.0 ml. aliquot of saliva and those calculated for the total of the specimen were subtracted from their respective control values. These mean differences were pooled in each group, and the "t" test applied at the 95 per cent confidence level gave the following results. (t = values, $t_{95} = \pm 3.00$.) Under the conditions imposed, only dry ice

TREATMENT PRIOR TO TITRATION	pH = 6		pH = 5	
	2 ML.	TOTAL SAMPLE	2 ML.	TOTAL SAMPLE
Deep-freeze for 18 hr.	−9.75	−2.27	+3.24	+3.00
Dry ice for 18 hr.	+1.3	+1.23	−0.41	−0.35
One hour at 25° C. then dry ice for 48 hr.	+11.53	+9.56	−9.06	−4.27

storage up to 18 hours gave results comparable with immediate titration values. With one exception, the buffering effect was more variable at pH 6.0 than at pH 5.0. In the major study, all specimens of saliva from the children to be tested for buffering ability were, therefore, promptly stored after collection in a portable dry ice cabinet and titrated within 18 hours to a pH of 5.0.

XVII. Periodontal Studies: Experimental Disease Production and Enzymology. March 21, Afternoon, Section C.

153. A Survey of the Bacterial Flora of the Periodontium in the Rice Rat.—*J. B. Macdonald, S. Socransky, and S. Sawyer, Forsyth Dental Infirmary, and Harvard School of Dental Medicine, Boston.* Fourteen 21-week-old rice rats, 9 with periodontal destruction and 5 with grossly normal periodontal structures, were sacrificed. The molar teeth and periodontium of one side were removed en masse and ground in broth. The resultant suspensions were cultured aerobically and anaerobically on horse blood agar, and anaerobically in Rogosa Veillonella medium. Forty-five representative cultures were derived by subculture to blood agar and heart infusion broth. Recurring strains were identified as follows: Enterococci—nonhemolytic, nonmucoid on sucrose agar, growth at 10° C., at 45° C., at pH 9.6, in 6.5 per cent NaCl and in 0.1 per cent methylene blue; coliforms—typical on EMB agar, and paracolon bacilli—late lactose fermenters, catalase and methyl red positive, indole and Voges Proskauer negative; Actinomyces—anaerobic, catalase negative, fermenting xylose, salacin, and raffinose, but not forming lactic acid; diphtheroids—catalase positive, black colonies on serum tellurite agar, no growth on lactobacillus agar; lactobacilli—catalase negative, growth on Rogosa agar but not tellurite agar, indole negative, nitrates not reduced, lactic acid from glucose; Actinobacillus—gram-negative microaerophilic coccobacilli, failing to produce indophenol oxidase or catalase, and variable in fermentation reactions; Staphylococci—facultative, catalase positive, creamy white colonies. Single strains of Bacteroides, Fusobacterium, Clostridium, and Bacillus species were also recovered. Spirochetes were not observed in dark-field examination of ground suspensions.

154. QUANTITATIVE STUDIES OF THE BACTERIAL FLORA OF THE PERIODONTIUM IN RICE RATS.—*S. Socransky, J. B. Macdonald, and S. Sawyer, Forsyth Dental Infirmary, and Harvard School of Dental Medicine, and A. M. Auskaps, Harvard School of Dental Medicine, Boston.* Eighteen 21-week-old rice rats were scored for periodontal disease according to the method of Gupta and Shaw, 1956. The molar teeth and periodontium of one side of the heads were weighed, and ground in broth. Dilutions determined in preliminary trials were plated in 0.1 ml. amounts on each of 3 plates in the following media: horse blood agar, Na azide agar, nutrient agar, EMB agar, Rogosa SL agar, and serum tellurite agar. The blood agar was incubated anaerobically for 5 days, other media aerobically for 3 days. Colony counts were made at ×10 magnification with a stereoscopic microscope. Total counts from growth on blood agar incubated anaerobically ranged from 3.6×10^7 to 1.7×10^{10} per total sample. Counts averaged 3.4×10^8 for 12 animals with periodontal scores of 57 or less, and more than 1.2×10^{10} for 6 animals with scores exceeding 100. Enterococci were the most numerous organisms, with counts ranging from 2.0×10^6 to 1.4×10^{10}. High counts, averaging 1.0×10^{10}, were associated with high periodontal disease scores. Actinobacillus counts ranged from 1.0 to 5.6×10^8 for the 4 rice rats with highest periodontal scores. Except for 1 animal, remaining Actinobacillus counts averaged 6.9×10^6. The only other regularly occurring organisms were diphtheroids, ranging in counts from 3.2×10^5 to 7.2×10^7, and unrelated to periodontal disease. Staphylococci, enterics, and lactobacilli appeared on plates from over half the animals, usually in the ranges 10^6 to 10^7, 10^4 to 10^5, and 10^3 to 10^6, respectively. Fusobacterium, although recovered only twice, numbered 1.2×10^9 in one animal.

155. THE EFFECT OF COLD AS A STRESSOR AGENT UPON THE PERIODONTIUM OF ALBINO RATS.—*Gerald Shklar and Irving Glickman, Tufts University School of Dental Medicine, Boston.* The effect of cold as a stressor agent on the periodontium was studied, using 70 young adult male albino rats, Wistar strain, aged 2 months and weighing 200 grams. Thirty-five animals were maintained at a constant temperature of 0° to 2° C. for experimental periods of 2 weeks (15 animals), 1 month (15), and 4 months (5). Thirty-five littermates kept at 25° C. served as controls. Changes occurred in the periodontium of the 2-week experimental group and more severely in the 1-month group. There was osteoporosis of alveolar bone characterized by a retardation in bone apposition so that, in severe cases, very little osteoid was present in areas where it is normally found. In less severe cases, bone apposition was found but reduced in amount. The normal distribution of osteoclasts was unaltered but the number of cells was reduced. The number of cells and fibers of the periodontal membrane appeared reduced and there was some modification in nuclear morphology. There was less evidence of cementum apposition. The gingival sulcus and epithelium were comparable to the controls. There was little evidence of change in the periodontium of the 4-month group.

156. THE EFFECTS OF AN ULTRASONIC FIELD ON PULP AND PERIODONTAL TISSUES OF RAT FIRST MOLARS.—*George W. Burke, Jr., Medical College of Virginia, Richmond.* A histologic evaluation was made of the effect of ultrasonic cavity cutting procedures on rats. A total of 36 animals was included in this experiment. Cavities were prepared in the right first molars with the ultrasonic method, using specially designed cutting tips approximately 0.5 mm. in diameter. The total cutting time was approximately one second. Control cavity preparations were made in the left first molars, utilizing conventional rotary

methods and small burs. All cavities were sealed with zinc oxide and eugenol. Animals were sacrificed following cavity preparation on Day 1 through Day 60. Whole maxillae were fixed and decalcified. Sections were cut at 7 μ and stained with hematoxylin and eosin. The majority of the experimental pulps and periodontal tissues showed evidences of histopathologic changes which we felt could be attributed to an ultrasonic field. These changes ranged from early degeneration of pulp, periodontal tissues, and surrounding alveolar bone to complete necrosis. In some instances, similar tissues of the adjacent second molar were involved. In a few cases, where less damage was apparent, the changes were metaplastic in character, manifested by deposition of bone among the periodontal tissues leading to ankylosis. Other effects were observed in red bone marrow, blood vessels, and regional salivary glands. None of the control teeth exhibited any unusual changes other than typical inflammatory reactions in the pulp following conventional methods of cutting.

157. Effects of Stress on the Periodontium of the Hamster.—*Om P. Gupta, University of Illinois College of Dentistry, Harry Blechman and Sigmund S. Stahl, Murry and Leonie Guggenheim Institute for Dental Research, New York University College of Dentistry, New York.* Sixty weanling hamsters were distributed into two groups comparable with regard to age, sex, and body weight. The 40 animals of the experimental group were subjected to the stress of ringing of bells and bright lights. The bells rang for 90 seconds every 10 minutes for a period of 24 hours. During the following 24 hours the animals were exposed to lights. During the third 24-hour period, the animals were not stressed. This cycle was carried on for 10 consecutive weeks. The 20 control animals were housed in a separate room away from the experimental group. All animals were kept in individual wire-bottom cages and were sacrificed after 10 weeks. The periodontal tissues were examined macroscopically and microscopically. The gross evaluation of the periodontal lesions was done by the method of Gupta and Shaw (Oral Surg., Oral Med. & Oral Path. 7: 727, 1956). The results showed no statistically significant difference in the number and extent of soft tissue lesions between the control and stressed animals. The stressed animals, however, showed a moderately significant increase in both the number and extent of calcified tissue lesions. The histologic studies showed widening of the bone marrow spaces and osteoporosis of the alveolar bone only in the stressed animals.

158. Further Observations on the Effects of Ovariectomy Upon the Periodontium of Experimental Animals.—*Irving Glickman and Giuliano Quintarelli, Tufts University School of Dental Medicine, Boston.* Two hundred and eighty female albino mice, R.A.P. strain, were divided into two age groups, 4 to 6 weeks and 1 year old. Seventy animals in each age group were ovariectomized and sacrificed at postoperative intervals up to 6 months. Nonoperated and sham-operated animals served as controls. The periodontium was studied microscopically in buccolingual sections in the mandibular first molar area. Absence of estrus was determined by microscopic examination of vaginal and uterine mucosa obtained at autopsy. In the alveolar bone, ovariectomy resulted in an inhibition in varied degrees of bone formation and resorption normally present in relation to the periodontal membrane in response to functional pressure and tension. In the periodontal membrane, the cellularity and fiber density appeared to be reduced. These changes occurred in both age groups, 2 to 3 months after operation, and persisted throughout the remainder of the 6-month experimental period.

159. THE EFFECT OF ORAL SOMATOTROPIC HORMONE INJECTIONS UPON GINGIVAL WOUNDS IN RATS.*—*S. S. Stahl, New York University College of Dentistry, New York.* Fifty-five male rats, weighing an average of 230 grams at the beginning of the experiment, were divided into three groups of 20, 15, and 20 animals, respectively. Group 1 received an experimentally created injury to the interdental papilla between the maxillary first and second molars. Ten of these animals received 0.5 mg. of STH in 0.1 c.c. of distilled water, buffered to a pH of 10, three times weekly into the site of injury. The remaining 10 animals received similar injections of the vehicle alone. Group 2, comprising 15 noninjured animals, received STH and vehicle injections (10 animals—STH injections, 5 animals—vehicle injections) into the interdental papilla between the two molars. Group 3 received oral injuries and injections as outlined for Group 1 and, in addition, were placed on a protein-free diet. The experimental period for all groups was 6 weeks. Gross observation indicated that animals, receiving oral STH injections alone, gained weight more rapidly (average weight gain = 79 grams ± 10.3) during the experimental period than did animals receiving injections of the vehicle (average weight gain = 50 grams ± 13.3). Animals receiving a gingival injury in addition to oral STH injections gained less weight (average weight gain = 59 grams ± 10.4) than did the hormone-treated, noninjured animals. Animals on a protein-free diet and receiving oral injections lost approximately half of their initial weight during the experimental period. STH injections did not alter the weight loss. Histologic observations of the site of injury showed a generalized gingival recession. Animals receiving STH injections exhibited apposition at the interdental alveolar crest in most specimens studied. Vehicle-injected control sites showed little evidence of crestal apposition. STH-treated, protein-deprived animals did not exhibit the alveolar endosteal osteoporosis and reduction in cellularity of the periodontal membrane seen in the vehicle-injected, protein-deprived rats, and some specimens showed slight osteogenesis at the interdental septum.

160. EFFECT OF EARLY LOSS OF FUNCTION ON MOLARS OF MICE.†—*Sidney A. Cohn, Division of Anatomy, University of Tennessee, Memphis.* Right upper molars of 127 albino mice, extracted at 25 days of age, were used to observe the long-term effect of early loss of function on teeth without antagonists. Four experimental animals and 3 intact controls were sacrificed every 50 days thereafter. Lower right jaws were removed and the molars studied grossly as well as microscopically. Macroscopic observations showed that little cuspal wear occurred on molars without function, while cusps of control animals were markedly abraded. In teeth lacking antagonists, there was an intense blackening and staining of the enamel. Histologic sections revealed that loss of function alone, without any associated infection, caused a remarkable and progressive resorption of interdental bone, leading to its almost complete disappearance by 400 days. During this period there was an extensive resorption of cementum, a disorientation and loss of fibers of the periodontal membrane, and an apical proliferation of the epithelial attachment. Only remnants of periodontal tissues, located between the root tips, remained in the interseptal region at 400 days. The massive apical deposition of cementum, characteristic of occluding teeth of mice, ceased after the first 100 days without function. These severe atrophic changes were interpreted as being due to the almost total lack of stimulation of the periodontal tissues by occlusal contact. In an attempt to verify this interpretation, first upper molars alone are now being extracted to allow some occlusal contact.

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161. Induction of Dental Caries and Periodontal Changes With Hydrogen Peroxide.—*Max Shapiro, Ved Brat, and Benjamin H. Ershoff, Dental Research Division, Western Biological Laboratories, Culver City, Calif.* The effects of H_2O_2 administered for 2 months in the drinking water as a 0.5, 1.0, or 1.5 per cent solution were determined on the teeth and periodontium of rats. Four groups of 20 animals were selected at weaning and were fed ad libitum a desiccated, natural food, noncariogenic ration (Rockland rat diet), plus either distilled water or water plus H_2O_2 in the concentrations indicated above. Growth was retarded in all rats administered the H_2O_2 solutions; the degree of retardation being proportional to the level of H_2O_2 administered. Animals were sacrificed after 60 days and sections prepared of the teeth and periodontal tissues. Sixty per cent or more of the rats, administered H_2O_2 at the 1.0 or 1.5 per cent level, exhibited extensive carious lesions involving the pulp. The carious lesion first appeared at the tip of the cusp. There were large areas of pulp necrosis with destruction of periodontal fibers and inflammation in the periapical area. Areas of severe root and alveolar bone resorption with tooth fracture were also noted. A resorption zone was noted at the lingual aspect of the upper incisor which involved the alveolar crest and lingual root surface with evidence of a repair process. Some of the above changes, although less marked, were also observed in rats administered the 0.5 per cent H_2O_2 solution. The secretory cells in the salivary glands were atrophic in the 1.0 and 1.5 per cent H_2O_2 groups. None of the above abnormalities was noted in the control group administered distilled water nor in rats administered distilled water but pair-fed in the H_2O_2 groups.

162. Action of Enzymes on the Periodontal Tissue of Rats.—*Genevieve D. Roth, University of Kansas City, School of Dentistry, Kansas City, Mo., and Robert J. Bruckner, Western Reserve University, School of Dentistry, Cleveland, Ohio.* Incisor teeth, with the periodontal membrane and bone intact, were taken from the maxillae and mandible of rats and fixed with ethylene oxide and/or formalin. An in vitro test was devised whereby tension could be developed in the periodontal membrane in situ. Teeth were suspended in a culture medium (pH 7), placed under varying degrees of tension, and inoculated with selected pure subcultures or with bacteria from the gingival sulci of patients with periodontal involvement. After incubation (3 days at 37° C.), the tissues were fixed, decalcified by chelation, and histologically stained with van Gieson, hematoxylin-eosin and Mallory stains. A second phase tested purified proteolytic enzymes under the same experimental conditions. No reaction was evident in experimental or control tissue pre-fixed in formalin. Tissue controls pretreated with ethylene oxide showed no changes. Bacterial cultures produced either no enzymes or enzymes incapable of bringing about cellular changes under the conditions of the experiment. Some tearing was evident in both experimental and control tissue and occurred more often at the periodontal attachment to the bone than at the cementum. Of the purified enzymes tested, trypsin 2 ×, trypsin 4 ×, collagenase, papain, and cathepsin caused varying degrees of destruction of the fiber elements. Urease was inactive.

163. (Withdrawn.)

164. Lysozyme Content of Human Gingiva and Various Rat Tissues.—*George W. Burnett, Susan Gouge, and A. E. Toye, Walter Reed Army Institute of Research, Washington, D. C.* Lysozyme occurs in tissues in varying quantities and it increases in response to irritation and inflammation. Under certain circumstances, lysozyme may be related to the development of ulcerative lesions. Since periodontal tissue is often subject to many irritants to which it responds

by inflammation, it was of interest to determine the lysozyme content of inflamed gingival tissue for comparison with that of other tissues. The lysozyme content of normal rat gingiva, spleen, kidney, and liver and inflamed human gingiva obtained from gingivectomies was determined according to the method of Lobstein and Fogelson (Quart. Bull. Northwestern U. Med. School 25: 89, 1951). The normal gingiva of 9 rats contained an average of 17,662 μg per gram of tissue in comparison to 370, 163, and 40 μg per gram, respectively, for kidney, spleen, and liver. The mean percentage lysozyme content of rat gingiva, kidney, spleen, and liver was, respectively, 1.76 per cent, 0.037 per cent, 0.016 per cent, and 0.0038 per cent. The mean lysozyme content of human gingival tissue from 14 gingivectomies was 1,493 μg per gram of tissue, and the mean percentage composition was 0.15 per cent.

XVIII. Chemical Studies and Hemodynamics. March 22, Forenoon, Section A.

165. Effect of Calcium and Phosphorus on Fluoride Absorption.—*Martin J. Wagner, Baylor University College of Dentistry, Dallas, and Joseph C. Muhler, Indiana University School of Dentistry, Indianapolis.* Fluoride metabolism has been studied principally by measuring the fluoride retained in the skeleton after its ingestion under various conditions. Few attempts have been made to measure the rate of fluoride absorption by the gastrointestinal tract or to determine its optimal site of absorption. This study was designed to measure the rate of absorption of fluoride by interrupting its absorption at various times following ingestion. Salts of calcium or phosphorus were used to interrupt absorption because of their known effect on fluoride utilization. Efficiency of absorption was measured by determining fluoride in the whole carcass. Fluoride given by stomach tube as a sodium fluoride solution was followed after 15, 30, or 60 minutes by solutions of either calcium chloride, sodium dihydrogen phosphate, or both salts. One group of animals received fluoride, calcium, and phosphate ions in one solution. The results of these studies indicate that the animals receiving a solution containing fluoride, calcium, and phosphate ions retained the smallest proportion of fluoride. When calcium and phosphate were introduced 15 minutes after fluoride ingestion, however, significantly more fluoride was retained when compared to the animals receiving the ions simultaneously. This suggests that a significant amount of fluoride had been absorbed in the 15-minute interval. These studies suggest that fluoride is rapidly absorbed by the gastric mucosa. Calcium and phosphorus may affect fluoride metabolism through their influence on fluoride absorption.

166. The Metabolic Distribution of 2-C^{14} 5:5 Diphenylhydantoin in the Ferret.—*P. N. Abadom, The University of Rochester, School of Medicine and Dentistry, Rochester.* The metabolic pathway of diphenylhydantoin in the female ferret was studied by the intramuscular administration of 19.3 μc per kilogram body weight, equivalent to 18.6 mg. of diphenylhydantoin. The animals were sacrificed at 1, 2, 4, 8, 16, and 24 hours, respectively, and tissues from the brain, salivary glands, plasma, red cells, gingiva, liver, kidneys, lungs, heart, spleen, muscle, fatty tissue, and gastrointestinal tract and contents were removed for radioactive analysis. The excretion of diphenylhydantoin was determined by radioactive analysis of urine, feces, bile, saliva, and expired carbon dioxide. The aim of the study was to determine if information gained in observing the metabolism of the drug could be correlated with the gingival hyperplasia induced in the ferret by experimental administration of diphenylhydantoin. Within 1 hour after the injection, the highest concentration of

diphenylhydantoin and metabolites were found in the contents of gastrointestinal tract, plasma, liver, and kidneys, respectively. Low concentrations were obtained from the brain, lungs, and heart. Still lower concentrations were found in the salivary glands, gingiva, and red cells. The radioactivity decreased in all organs to within zero limits in 24 hours. The excretion of diphenylhydantoin occurred mainly in the urine, and the presence of high concentration in the contents of the gastrointestinal tract showed that biliary excretion took place. The excretion of diphenylhydantoin occurred in the expired CO_2, indicating that the hydantoin ring was broken to some extent in the course of metabolic degradation.

167. Effect of Fats Upon Cholesterol, Fluorine Storage, and Carbohydrate Metabolism.—*David Bixler and Joseph C. Muhler, Indiana University School of Dentistry, Indianapolis.* A total of 150 1-day-old chicks were divided at random into five experimental groups. All animals in Group I, the controls, were fed a basic diet composed of (in per cent) sucrose, 75; casein, 18; salt mixture, 4; vitamin mixture, 1; and cellulose, 2. Group II had cottonseed oil substituted for sucrose such that the diet contained only 55 per cent sucrose but 20 per cent cottonseed oil; the other dietary constituents were not changed. Similarly, Groups III, IV, and V had oleic acid, stearic acid, and lard substituted, respectively, at the same level as the cottonseed oil for the sucrose. Serum cholesterol was determined weekly, and after 8 weeks all animals were sacrificed. Representative soft tissues were removed for fluoride analysis, and the adrenal and thyroid glands with a portion of the liver were weighed and fixed for histologic study. Serum cholesterol levels were consistently higher in the fat-supplemented groups than in the controls. No significant differences in fluorine storage of soft or skeletal tissue were noted. Adrenal glands of the fat-fed animals were significantly heavier than the controls, suggesting a stimulation of gluconeogenesis, and liver sections from these animals indicated an increased glycogen storage. The control animals on the high carbohydrate regimen had a considerable deposition of intracellular fat in the liver with little or no glycogen present. The significance of these findings will be discussed.

168. Formation of Collagen Hydroxyproline In Vivo.—*Ernest Hausmann, The University of Rochester, School of Medicine and Dentistry, Rochester.* Previous studies by Stetten (J. B. C. 181: 31, 1949) and S. F. Jackson and associates (J. Biophys. & Biochem. Cytol. 3: 913, 1957) showed that protein-bound hydroxyproline is mainly derived from proline, hydroxylated when peptide-bound. The present study indicates that proline is hydroxylated in a proline-containing peptide having a molecular weight less than 12,000-15,000, rather than in a fully formed tropocollagen molecule with an excess of proline and deficient in hydroxyproline. Guinea pigs bearing carageenin-induced granulomas were injected intraperitoneally with C^{14} proline. The ratio of the specific activities of proline/hydroxyproline from purified collagen of the granulomas was found to be 1.1. An ultrafilterable hydroxyproline-containing peptide was obtained from the granulomas.

169. Histochemical Demonstration of Cytochrome Oxidase in Oral Tissues.—*M. S. Burstone, National Institute of Dental Research, National Institutes of Health, Bethesda.* In the past, the "Nadi" reaction of Ehrlich, employing alpha naphthol and an N-alkylamine, has been used for the histochemical demonstration of cytochrome oxidase. Unfortunately, this technic results in unstable and diffusible dyes, and thus limits definitive localizations. N-alkyl amines have also been found to be toxic to the enzyme by Borei. The present

study utilized new technics for the demonstration of the enzyme, and employed new complex naphthols as well as compounds with reactive methylene groups as substrates (J. Histochem. & Cytochem., in press). The new substrates included 2,4-dichloro-5-p-toluenesulfonyl amino naphthol, phenyl 1-hydroxy-2-naphthoate, 1-hydroxy-2-naphthoic acid, 1-phenyl-3-methyl pyrazolone, 1-(4′-phenoxy-3′-sulfophenyl) 3-heptadecylpyrazolone-5, 2-cyanoacetyl coumarone, and α, α'-terephthaloylbis (5-chloro-2,4-dimethoxyacetanilide). Hitherto undescribed localizations of the enzyme in muscles of mastication, including tongue, salivary gland ducts, and gingiva, were found. The reactions were run on unfixed frozen sections. An alternate technic which employed frozen-dried specimens was also evolved. The reaction was inhibited by cyanide, azide, and sulfide. On the other hand, cytochrome *c* was found to augment staining. Sections pre-incubated in saline revealed little or no staining due to loss of cytochrome *c*. The addition of cytochrome *c* to the incubating solution restored the reaction.

170. CHOLESTEROL CONTENT OF SOME BOVINE ORAL TISSUES.—*James J. Stickley and Alton K. Fisher, College of Dentistry, State University of Iowa, Iowa City.* Few data are available concerning cholesterol content of various oral tissues. Hodge reported total sterol of human gingiva as 0.2 per cent. Hess, Lee, and Peckham determined cholesterol for human enamel and dentin as 0.008 and 0.024 per cent, respectively. The present study employed the spectrophotometric method of Foldes and Wilson in determining the cholesterol content of normal bovine dental pulp, gingiva, and parotid gland. Bovine Achilles' tendon was also analyzed to provide comparative information for connective tissue. The quantitative values presented are mean percentages of fresh tissue weight, and are based on analyses of 30 samples of each tissue, except in the instance of Achilles' tendon where 22 samples were used. Pulp tissue was graded as Embryonic, Young, or Mature depending on whether tooth development had not yet advanced to beginning root formation, root formation was in progress, or had been completed. The cholesterol content of Embryonic, Young, and Mature dental pulp was 0.067, 0.069, and 0.078 per cent, respectively. That of Achilles' tendon was 0.035 per cent. The increasing value of pulpal cholesterol with increasing age and fibrous character is not understood. The cholesterol content of gingiva was 0.108 per cent. That of parotid gland was 0.242. Differences in analytic methods and well-substantiated species differences did not permit exact comparisons of the present limited data for oral tissues.

171. RADIOLOGIC, BIOCHEMICAL, AND HISTOCHEMICAL STUDIES OF RADIOACTIVE ASCORBIC ACID IN THE GUINEA PIG.—*S. H. Yale, H. Jeffay, and E. Wach, University of Illinois, Chicago, and C. I. Mohammed, University of Puerto Rico, San Juan, P. R.* In normal and scorbutic guinea pigs injected with radioactive ascorbic acid, radioactivity was localized in the radioautographs of pulp, periodontal membrane, alveolar periosteum, cortical and supporting bone. The vitamin was identified, histochemically, adjacent to the odontoblastic layer of the pulp, in the interlobal and interlobular connective tissue of the submaxillary gland. By chemical analysis, we were able to correlate the radioautographic and histochemical findings by identifying the same pattern of distribution of radioactivity in the tissues described above. All of our technics failed to reveal any ascorbic acid in enamel or dentin. In addition, we found that oral tissues showing specific pathology had the highest concentration of radioactive, nonascorbic acid compounds, whereas the liver did not show as profound a pathologic change and had the lowest concentration of these radioactive nonascorbic

acid compounds. We concur with other reports in the literature that the amount of radioactive ascorbic acid incorporated into the various tissues is approximately the same in the scorbutic as in the normal animal. Further examination of radioautographs showed a difference in the pattern of distribution of radioactivity within specific tissues when the normal and scorbutic jaws were compared. Our findings of distribution of ascorbic acid in the oral tissues may be a reflection of the physiologic utilization of ascorbic acid to repair the damage occurring during the scorbutic period, and/or an expression of the growth function in the normal.

172. An Electromagnetic Blood Flow Meter for Measuring Small Volume Flows.—*J. G. Bishop, H. L. Dorman, and R. A. Huffines, Baylor University College of Dentistry, Dallas.* Previously designed electromagnetic blood flow equipment has been difficult to adapt to blood flow measurements in the oral areas. An electromagnetic blood flow meter was developed which retains stability and yet has sufficient gain to accommodate small volume blood flows which are found in the oral regions. Maintenance of a constant phase relationship is essential for accurate flow measurements with an electromagnetic blood flow meter. Two precision micropots were incorporated into the present system in order to provide precise control of bucking voltage and phase. Continuous monitoring of phase was accomplished by driving the horizontal plates of a cathode ray oscilloscope with voltage in phase with cannula excitation. The vertical sweep was driven by the amplified resultant of the cannula and bucking voltage with a 180° phase difference. Rectification of the amplified cannula and bucking voltage resultant allowed flow recording on an ink-writing oscillograph. Construction of different size cannulae allowed measurements of widely varying volume flows with the minimum measurable flow rate approaching zero. In general, highest cannula voltages were developed with smallest pick-up probes, hence, large diameter probes were avoided in small flow measurements. Instrument calibration was accomplished by collecting blood from the open distal side of the cannula and measuring the deflection at various flow rates. Calibration was linear, within ± 5 per cent in all ranges above 1 c.c. per minute.

173. A Comparison Between Maxillary and Mandibular Hemodynamics.—*D. R. Redden, H. L. Dorman, J. L. Matthews, and J. G. Bishop, Baylor University College of Dentistry, Dallas.* This group has previously reported blood flow, blood pressure, and peripheral resistance in the mandibular arteries of mongrel dogs. The purpose of this investigation was to compare maxillary and mandibular hemodynamics. A minimum of 9 large healthy dogs was used for each variable; the animals were anesthetized with sodium pentobarbital, 33 mg./Kg., and given 1 mg./Kg. of heparin. Polyethylene tubing was used to cannulate the mandibular artery proximal to the mandibular foramen and the maxillary artery distal to the mandibular branch. Blood flow was continuously recorded by use of a simplified electromagnetic blood flow, meter, and pressure was simultaneously recorded by use of a Statham strain gauge, Brush amplifier, and a Brush recorder. Mean control blood flow was approximately 3 times greater in the maxillary than in the mandibular arteries. Pressure was not significantly different. Mean peripheral resistance was approximately 3 times higher in the mandibular artery. One-tenth microgram of epinephrine and 1 μg of acetylcholine produced vasoconstriction and vasodilatation in each vascular bed. Radiographic analysis, during infusion of Thorotrast, delineated the areas supplied by the mandibular and maxillary arteries.

174. THE EFFECTS OF TEMPERATURE CHANGES ON MANDIBULAR HEMODYNAMICS.—*E. E. Moore, J. G. Bishop, H. L. Dorman, and J. L. Matthews, Baylor University College of Dentistry, Dallas.* Cardiovascular responses to temperature changes in the oral region were ascertained in the mandibular artery of anesthetized dogs. The animals were anesthetized with 33 mg./Kg. sodium pentobarbital, and 1 mg./Kg. heparin was used as an anticoagulant. A minimum of 6 dogs was used for each experimental procedure, each dog serving for its own control and for multiple assays. Direct cannulation of the mandibular artery permitted simultaneous recordings of blood flow and blood pressure. Flow was measured by use of an electromagnetic blood flow meter, and pressure with a Statham transducer, Brush analyzer, and Brush recorder. Temperature was determined by thermocouples placed lateral to the mandible, medial to the madible, and in the rectum. Temperature variations to the oral region were induced by application of an infrared lamp and/or cold packs. Application of heat, sufficient for producing a rise of 2.2° C. in tissue lateral to the mandible, increased blood flow approximately 44 per cent. Application of cold, which decreased the temperature in the same area by 6° C., decreased flow approximately 74 per cent. The temperature changes preceded blood flow variations in such a manner that the increased flow rate persisted for a time after the temperature returned to control level.

XIX. SALIVARY GLANDS. MARCH 22, FORENOON, SECTION B.

175. EFFECT OF SELECTIVE SALIVARIADENECTOMY ON CARIES AND SEVERAL SALIVARY PROPERTIES.—*Sam Rosen, Charles J. Sylvester, Carl A. Hoppert, and H. R. Hunt, Michigan State University, East Lansing.* The development of caries-resistant and caries-susceptible lines of rats by Hunt and Hoppert has demonstrated that heredity is a factor in the production of dental caries. Saliva may contain one or more factors influencing dental caries since the removal of the major salivary glands hastened the production of caries. When the submaxillary and sublingual glands were extirpated, caries developed more rapidly. However, when only the parotid ducts were removed, the development of caries was delayed. Several properties of pilocarpine-stimulated saliva from the parotid gland and of the combined salivas from the submaxillary and sublingual glands from both caries-resistant and caries-susceptible rats were studied. The pH and buffer values were approximately the same for all salivas. The rate of flow from submaxillary and sublingual saliva was about twice that obtained from parotid saliva. The relative viscosity of submaxillary and sublingual saliva was greater than that of parotid saliva. More than 99 per cent of the salivary amylase activity originated from the parotid glands. There was little or no difference between caries-resistant and caries-susceptible rats for pH, rate of flow, and buffer value of saliva. In caries-resistant rats, the relative viscosity was higher and the amylase activity was lower.

176. EFFECT OF THIOCYANATE ON THYROID, SALIVARY GLANDS, AND CARIES INCIDENCE.—*William G. Shafer, Joseph C. Muhler, and Patricia G. Clark, Indiana University, Indianapolis.* Studies in these laboratories have shown a relationship between thyroid gland function, the histologic structure of the salivary glands, and experimental dental caries in the rat. These studies were extended to investigate the effect of a known goitrogen, potassium thiocyanate, on the dental caries incidence and on two salivary gland functions, flow and viscosity. A series of 122 male weanling rats was divided into 5 groups: (1) controls, (2) animals given 10 to 100μg of dl-thyroxine per day per animal, (3) animals receiving 0.25 per cent potassium thiocyanate in the drinking water,

(4) animals given 0.1 per cent propylthiouracil in the diet, and (5) animals receiving thyroxine and propylthiouracil at the above levels. A stock corn cariogenic diet and fluoride-free distilled water were available ad libitum. The mean number of carious lesions for the respective groups described above were: (1) 10.1, (2) 7.8, (3) 10.5, (4) 11.3, and (5) 9.0. The thyroid glands of animals receiving thiocyanate were greatly enlarged and similar in many respects to those animals receiving thiouracil. However, the thiocyanate-treated animals did not show the changes in the submaxillary glands that are characteristic of thiouracil-treated animals; namely, a reduction in size and cytoplasmic content of the granular tubules. Sixteen rats were also divided into 3 groups: (1) controls, (2) 0.1 per cent propylthiouracil in the diet, and (3) 0.1 per cent potassium thiocyanate in the drinking water. After 30 days, salivary flow and viscosity were determined. These were: flow—(1) 1.6 ± 0.5 ml., (2) 0.7 ± 0.4 ml., and (3) 2.8 ± 1.0 ml.; viscosity—(1) 13.6 ± 6.0 sec., (2) 18.1 ± 4.3 sec., and (3) 8.8 ± 1.6 sec. The data indicate that an exception exists to the general findings that decreased thyroid function results in an increased dental caries experience and decreased salivary flow.

177. Effect of Thyroid Hormone and Testosterone on Salivary Gland Phosphorous and Nucleic Acid Content.—*Donald E. Flieder, St. Louis University School of Dentistry, St. Louis.* Male weanling rats of the St. Louis University strain were divided into three groups. One group (11) was fed desiccated thyroid (1 gram per kilogram feed), the second group (9) was injected intramuscularly witih testosterone propionate (5.5 mg.) in oil at weekly intervals, and a third group served as controls. Nothing was known concerning the caries incidence of these animals. After 88 days the animals were sacrificed and homogenates prepared of the salivary glands. These homogenates were then analyzed by the Schmidt-Thannhauser-Schneider technic. The following determinations were performed: inorganic phosphorus(IP), organic phosphorus(OP), phospholipid(PL), ribonucleic acid(RNA), desoxyribonucleic acid(DNA), and residual protein(RP). Ratios were constructed using DNA as the parameter. There was no statistically significant difference in the RNA/DNA ratio between the control and experimental groups. The testosterone injected animals showed a significant increase in the IP/DNA and RP/DNA ratios over the control group. The thyroid-fed animals showed a decreased PL/DNA ratio, but did not differ significantly from the control group in any other respect.

178. Body Weight and Metabolic Rate of Sialoadenectomized and Intact Rats.—*John Haldi, Winfrey Wynn, and Ennis Goettee, Emory University, Atlanta.* Evidence has been accumulating in the literature to indicate that the salivary glands may have an influence on metabolic processes. Retardation in growth and changes in the adrenals, testes, and uteri have been observed as a result of sialoadenectomy. The present experiments were undertaken to determine (1) gain in weight, and (2) metabolic rate of sialoadenectomized and intact animals on the same food intake. The intact animals were subjected to a sham operation in which the salivary glands were exposed and teased gently without removal. Thirty pairs of animals were fed 60 days by the paired-feeding method. The intact males gained 57 grams more and the females 39 grams more in body weight than their sialoadenectomized littermates. The sialoadenectomized males required 1.3 grams and the females 1.6 grams more food for each gram gain in body weight than their intact littermates. Comparable results were obtained on 5 pairs of animals fed by stomach tube throughout the experiment. The metabolic rate apparently was at a higher level in the sialoadenectomized animals. In the final days of the experiment, the oxygen consumption

per gram of body weight was 10 per cent higher in the sialoadenectomized animals than in the intact controls. This study is being extended to determine the oxygen consumption at different time intervals over the entire experiment.

179. FREE 17-HYDROXY CORTICOSTEROIDS IN HUMAN PAROTID SECRETION.—*Ira L. Shannon, and John R. Prigmore, School of Aviation Medicine, Randolph AFB, Texas.* Utilizing the method of Peterson and his co-workers, parotid fluid from healthy young male adults was analyzed for free (unconjugated) 17-OHCS under varying experimental conditions. The concentration in normal subjects was almost invariably under 5.0 gamma per cent (normal serum mean value 14.0 gamma per cent). Single oral dosage of 50 mg. of synthetic analogs of hydrocortisone produced at least three- to fourfold increases in the steroid content of parotid fluid during a 7-hour experiment. These values were parallel to serum steroid increases in these subjects. These findings suggest the use of parotid fluid steroid values as an indicator of adrenocortical status and, possibly, as an indicator of stress.

180. COMPARATIVE ENZYME CONCENTRATIONS OF SUBMAXILLARY AND PAROTID SALIVAS.—*Basil L. Henriques and Howard H. Chauncey, Tufts University School of Dental Medicine, Boston.* The object of this study was to determine and compare hydrolytic enzyme activity in the secretions from the parotid and submaxillary glands. Saliva samples were collected from 30 fasting individuals. The stimulus, 3 grams of sweetened chicle, was renewed every 5 minutes during the collection period. Parotid saliva was collected with vacuum cups. A modification of the Schneyer appliance was used to collect the submaxillary secretion. The acid phosphatase, total esterase, and pseudocholinesterase levels were determined using esters of beta-naphthol. Beta-glucuronidase activiity was measured with phenolphthalein glucuronic acid. The ranges, means, and dispersion values for each of the four hydrolytic enzymes measured are presented in Table I.

TABLE I

	PAROTID			SUBMAXILLARY		
	RANGE	MEAN	S.D.	RANGE	MEAN	S.D.
Enzymes						
Acid phosphatase*	0.13 - 1.38	0.58	0.268	0.19 - 1.20	0.55	0.288
Total esterase*	0.049- 0.101	0.065	0.0265	0.059- 0.455	0.118	0.078
Cholinesterase*	0.011- 0.131	0.064	0.0346	0.0 - 0.116	0.045	0.028
B-glucuronidase†	12.12 -150.00	81.56	39.70	5.88 -123.52	54.28	35.2

*Measured in Seligman units.
†Measured in Fishman units.

The mean acid phosphatase levels of the parotid and submaxillary gland secretions were quite similar in magnitude. However, a marked difference existed between the total esterase, pseudocholinesterase, and beta-glucuronidase levels.

181. OXIDATIVE METABOLISM OF SALIVARY GLANDS.—*Philip Person, Rose Marie Schneider, and Sylvia Scapa, VA Hospital, Brooklyn.* Indophenol blue synthesis via the Nadi reaction was used to determine the relative oxidative capacities of various cell types in rat salivary glands. Fresh frozen submaxillary, sublingual, and parotid glands were used. Sections were cut at 40 μ. In all glands, tubules and ducts synthesized indophenol blue at a much more rapid rate than did the acini. Ducts of the sublingual glands were invariably most active of the three glands studied. Next in activity were the submaxillary serous tubules and ducts. Those of the parotid gland were most variable in

activity. The submaxillary and parotid acini were most active of the acinar structures. Sublingual gland acini were least active. Cyanide and azide reversibly inhibited both tubular and acinar indophenol blue synthesis. Formalin irreversibly destroyed the ability of ducts and tubules to form indophenol blue. In contrast to the ducts and tubules acini, especially those of the submaxillary and parotid gland, retained their pigment-synthesizing capacity following treatment with formalin. It would thus appear that there are two separate mechanisms for the oxidative synthesis of indophenol blue in the salivary gland. The first, exhibited by tubules and ducts, appears to be mediated via the usual respiratory enzyme systems. The second mechanism survives treatment which would destroy most respiratory enzymes and is found only in the acini.

182. Hydrolytic Enzymes of Mammalian Salivary Glands.—*Giuliano Quintarelli and Howard H. Chauncey, Tufts University School of Dental Medicine, Boston.* The localization of certain hydrolytic enzymes (acid phosphatase, nonspecific esterases, β-galactosidase) was ascertained in the major salivary glands of rats, rabbits, sheep, cow, swine, and dog. Tissue samples were obtained within 30 minutes after sacrifice. Fixation was in 7.4 per cent formalin solution for 24 hours at 4° C. The tissues were then washed with running tap water for 2 hours and embedded in gelatin. Sectioning was done on a freezing microtone and the enzymes were localized using esters of beta-naphthol. Nonspecific esterases were demonstrated using a simultaneous coupling procedure. Postcoupling methods were used for acid phosphatase, β-D-galactosidase and β-glucuronidase. The findings, thus far obtained, indicate that the enzyme activity is confined to the cytoplasm and intimate structure of the serous acini and ducts. Nuclei were never observed to contain activity. Similarly, adipose tissue, blood vessels, and connective tissue were generally negative. Positive structures exhibited differences in reactivity depending on the enzyme demonstrated, species examined, and type of tissue.

183. Formation of Certain Vitamins in Saliva.—*T. Shiota, M. N. Disraely, and M. Caplow, National Institute of Dental Research, U. S. Department of Health, Education and Welfare, Bethesda.* An investigation was carried out in order to determine (1) the levels of folic acid, pantothenate and pyridoxine-like compounds in saliva, (2) the effects of incubation on the levels of these vitamins, and (3) the possible role played by certain oral bacterial on vitamin levels in saliva. Paraffin-stimulated saliva, and sterilized saliva inoculated with pure cultures of streptococci, lactobacilli, and veillonellae were incubated with and without glucose in order to follow changes in vitamin levels and bacterial population and to relate these changes to each other. Vitamins were identified by paper chromatography and determined by microbiologic assay. Chromatography of control saliva revealed traces of free folic acid and a compound similar to coenzyme A. Chromatograms of incubated salivas showed compounds similar to folic acid, citrovorum factor, an unidentified compound which migrated intermediate to folic acid and citrovorum factor, pyridoxine, pantothenate, and coenzyme A. The folic acid, pyridoxine, and pantothenate activities in the various salivas were as follows: 0.80, 0.99, and 785 mμg per milliliter in the control; 1.10, 5.50, and 1,405 mμg per milliliter upon incubation, and 3.60, 11.50, and 778 mμg per milliliter, respectively, upon incubation witih glucose. The concomitant bacterial changes which occurred in inoculated salivas indicated that the lactic acid bacteria could be active in increasing the levels of pyridoxine and folic acid-like compounds in saliva and that the fusobacteria and veillonellae could be active in increasing the level of pantothenic acid-like compounds.

184. CHANGES IN THE INTRALOBULAR DUCT WITH LIGATION.—*Theodore E. Bolden, Seton Hall College of Medicine and Dentistry, Jersey City, N. J.* The diameter of the lumen, the height of the lining cells and the diameter of the intralobular duct were measured in sections of the submaxillary glands of male albino rats. The excretory ducts of the right submaxillary glands were ligated for 1, 2, 3, 5, 7, and 9 days. The left glands were not ligated. Thirty-nine specimens, 15 nonligated (patent) and 24 duct-ligated glands, were used. Measurements were made of 10 ducts in each specimen. One intralobular duct, cut in cross section, was selected in each of 10 different fields. All measurements were made with an eye piece micrometer and recorded in microns. The diameter of the lumen and the diameter of the intralobular duct increased with ligation, while the height of duct lining cells decreased. The lumen of intralobular ducts of patent glands had an average diameter of 6.7 μ (range 4.8 to 8.1 μ) as compared to 11.2 μ (range 8.2 to 16.2 μ) for the lumen of ducts of ligated glands. Cells lining intralobular ducts averaged 14.5 μ in height in patent glands and 12.6 μ in ligated glands. The range was from 13.5 to 16.5 μ and 11.1 to 14.5 μ, respectively. Patent intralobular ducts had an average diameter of 35.9 μ (range 32.7 to 39.4 μ). Ligated intralobular ducts had an average diameter of 39.2 μ (range 30.2 to 47.0 μ).

XX. PULPAL AND ENDODONTIC STUDIES. MARCH 22, FORENOON, SECTION C.

185. HEALING OF PULPITIS UNDER A PRESSURE DRESSING.—*V. Kalnins, College of Dentistry, Western Reserve University, Cleveland, and H. E. Frisbie, University of California, School of Dentistry, San Francisco.* The pulp tissue of 53 roots of 25 single and 14 multirooted teeth of young dogs was examined by histologic and roentgenologic methods. All the pulps were exposed and left open for 2 to 5 days; thereupon 41 pulps were treated with calcium hydroxide paste for 3 to 18 days. The paste used was a commercial preparation composed chiefly of calcium hydroxide with 1 to 2 per cent strontium salts added (x-ray contrast medium). In order to thicken the paste and reduce its necrotizing effect, microcrystalline sulfathiazole was added to make up 50 to 80 per cent of the total. The wound was cleaned with 3 per cent solution of H_2O_2, and the paste was pressed onto the pulp with a cotton pellet, whereby total compaction of the coronal pulp tissue was obtained. The cavities were subsequently filled with amalgam or phosphate cement. All 4 untreated (control) pulps of single-rooted teeth showed more or less advanced pulpitis. In multirooted control teeth, pulpitis was present in 5 and necrosis present in 3 pulps. After a 3-day treatment, the successful cases showed either freedom from inflammation or a residual pulpitis. This latter condition had not disturbed the beginning of dentinal bridge formation. With longer treatment, pulpitis was absent and a bridge established. The failures showed advanced pulpitis or necrosis. Seventeen out of 21 pulps of the group of treated single-rooted teeth, and 9 out of 20 pulps of the group of treated multirooted teeth were considered successful.

186. EVALUATION OF MEDIA FOR ROOT CANAL CULTURE.—*William A. Nolte, The University of Texas, Dental Branch, Houston.* Four different culture media were evaluated for the purpose of determining the effect of 0.1 per cent and 0.2 per cent agar on growth sensitivity for a strain each of fusobacterium, streptococcus, lactobacillus, Actinomyces, and Candida. Tubes of Brewer, trypticase soy, brain heart infusion and microinoculum media, with and without added agar, were heated to 100° C. for 5 minutes, cooled to 45° C. and inoculated with 0.1 ml. of different dilutions of the respective test microorganisms. The dilutions were prepared from 24-hour broth cultures. All tests

were incubated at 37° C. for 5 days before being considered negative. Tests of the different media, without added agar, showed Brewer's medium to be most sensitive in detecting the fusobacterium and the lactobacillus. For the Actinomyces, microinoculum and trypticase soy were best and equally sensitive, and for Candida, trypticase soy was most sensitive. Each of the four media appeared to be equally sensitive for the streptococcus organism. The addition of agar increased the sensitivity of trypticase soy, microinoculum and brain heart infusion for the strains of fusobacterium and lactobacillus. Added agar increased the sensitivity of Brewer's medium for the Actinomyces. Added agar appeared to have no effect on increased sensitivity of any of the media for either the streptococcus or the *Candida* cultures. The addition of 0.1 per cent agar appeared to be as effective in increasing sensitivity of the media as 0.2 per cent agar. Trypticase soy plus agar was the most sensitive of all media tested.

187. Contamination of Endodontic Cultures with Para-aminotoluene Sulfonamide.—*B. F. Gurney and E. J. Best, Loyola University School of Dentistry, Chicago.* The purpose of this report is to show the relation between carry-over of residual para-aminotoluene sulfonamide (PATS) and culture growth inhibition when PATS is used as the endodontic antiseptic. Sterile brain heart infusion broth solutions were made up at concentrations of 10 mg. per cent, 1 mg. per cent and 0 mg. per cent of PATS, at pH 6. Similar solutions were made up at pH 7, 7.4, and 8. These solutions were inoculated with endodontal bacteria in a manner similar to that used in clinical practice. After 3 days' incubation at 38° C., all of the bacterial samples (10) showed growth in the presence of 10 mg. per cent PATS, a value believed to be considerably higher than any possible contamination. In addition, 257 negative clinical cultures were obtained from the Department of Endodontia, and inoculated in the same fashion as used previously. After 3 days' incubation, 252 showed bacterial growth. The remaining 5 cultures showed growth after reinoculation. From this evidence we conclude that 98 per cent of all cultures, taken and treated in the usual simple fashion, will yield accurate results.

188. Pathogenicity of Corynebacterium Isolated From Pulp Canals.—*Lucile S. Smith, Paul R. Thomassen, and John K. Young, College of Physicians and Surgeons, School of Dentistry, San Francisco.* The virulence of fourteen strains of Corynebacterium, isolated from nonexposed pulp canals of human teeth, was tested in normal mice and in both normal and irradiated rabbits. One-tenth milliliter quantities of brain heart infusion-yeast extract broth cultures, incubated 72 hr. at 37° C., were inoculated intraperitoneally into normal mice. No signs of toxicity were observed. In some of the mice, however, pustules formed at the site of inoculation through the peritoneal wall. Encapsulated lesions containing a purulent exudate were demonstrated in hematoxylin-eosin stained sections from these sites. When 0.1 ml. quantities of the 72-hour cultures were injected intracutaneously into rabbit abdomens, lesions developed which reached their maximum size in 48 to 72 hours. These lesions consisted of definite raised papules surrounded by red macules, and resolved, without draining, in periods ranging from 4 to 18 days. When the organisms were injected into the abdominal skin of rabbits whose abdomens had been irradiated (160r of x-ray divided into 16 doses distributed equally over the surface of the shaved abdomen), much larger lesions developed. These were fiery red papules with sharply raised edges and reached a maximum diameter in 72 hours. These lesions underwent spontaneous external

drainage. Resolution occurred from 20 to 40 days after inoculation. Hematoxylin-eosin stained sections showed that the lesions in the skin of non-irradiated animals were discreet abscesses, while those in the irradiated animals were poorly circumscribed, multiple, and extended over a much wider area.

189. EFFECTS OF SONIC VIBRATION ON RESPIRATION IN BOVINE DENTAL PULP.—*James H. Belding, Alton K. Fisher, and Edward M. Braley, College of Dentistry, State University of Iowa, Iowa City.* The introduction of high speed cutting and ultrasonic technics in cavity preparation has aroused interest in the possible harmful effects of these methods on the dental pulp. Investigation of these possibilities has been confined largely to histopathologic examinations in which subtle physiologic alterations are not always apparent. In the present study, pulps of normal bovine molars were cut into control and experimental portions so that individual organs could provide comparable data free of gross physiologic variations. The experimental tissues were subjected to vibration in the frequency range of 9 to 10 kc., the Raytheon sonic oscillator being used for this purpose. Twenty-two samples were exposed to this vibration for 3 minutes, 22 samples for 5 minutes, 21 samples for 10 minutes, and 18 samples for 15 minutes. After vibration, the experimental tissue and its control were sliced and their rates of oxygen consumption were measured by the direct method of Warburg over hourly periods for 4 consecutive hours. QO_2 values were calculated for each experimental sample and its corresponding control sample for each hourly period. Differences between the two values were expressed in terms of percentage of the control QO_2. Respiratory activity measured during the first hour was depressed by 11.5 per cent when the tissue was vibrated for 3 minutes, but only 2.8 per cent when vibrated for 5 minutes. Respiration was scarcely disturbed in tissue vibrated for 10 minutes (1.5 per cent), but tissue subjected to vibration for 15 minutes showed a respiratory depression of 32.6 per cent. After the first hour, the tissues vibrated for 3 and 5 minutes showed a larger depressant effect, while the tissue vibrated for 10 minutes respired at a rate almost equal to the controls. The tissue vibrated for 15 minutes appeared to have recovered slightly after the first hour and continued to respire at a rate approximately 28 per cent less than that of the controls for the remaining 3 hours.

190. EFFECTS OF PROCAINE CONCENTRATION ON RESPIRATION IN BOVINE DENTAL PULP.—*Christian Schwabe and Alton K. Fisher, College of Dentistry, State University of Iowa, Iowa City.* An earlier preliminary study indicated that procaine hydrochloride depressed respiration in bovine dental pulp. The present report presents the observed changes in respiratory rates in normal bovine dental pulp slices over 5-hour periods when suspended in procaine hydrochloride solutions in concentrations of 4, 2, 1, 0.5, and 0.25 per cent, respectively. Rates of oxygen consumption were measured by the direct method of Warburg. The suspending solution was Krebs-Ringer-phosphate buffered to pH 7.4, to which the procaine hydrochloride was added in the case of the experimental tissues. Each experimental sample of pulp tissue was provided with a control sample cut from the identical organ. QO_2 values were calculated for each sample for each hourly period. Results were expressed as percentage of normal QO_2, the control sample QO_2 value being 100 per cent. The effects of 4, 2, 1, 0.5, and 0.25 per cent solutions were tested with 23, 24, 23, 24, and 16 samples of tissue, respectively, and an equal number of control samples. At the end of the first hour, the 4, 2, 1, 0.5, and 0.25 per cent procaine solutions had depressed the rate of oxygen consumption by 71.9, 31.4, 29.5, 11.3, and 1.9

per cent, respectively. By the end of the fifth hour the depression had increased to 91.8, 69.8, 69.0, 46.9, and 30.5 per cent of normal, respectively. The rate of decline over the 5-hour periods was essentially parallel for all concentrations and fairly uniform throughout the period. The effects of the 1 and 2 per cent concentrations of procaine hydrochloride were almost identical. The extremely low level of oxygen consumption after the second hour in samples exposed to 4 per cent concentrations might be attributable to auto-oxidation. It is suggested that 4 per cent concentrations of procaine might have a lethal effect in 2 hours.

191. Rat Connective Tissue Reactions to Implants of Pulp Capping Materials.—*Ralph E. McDonald, Hala A. M. Zawawi, and David F. Mitchell, Indiana University School of Dentistry, Indianapolis.* The irritational properties of commonly used commercial cavity lining and pulp capping agents were studied. Twelve materials, Cavit, Serocalcium Paste, Cargenol, Gardinier Sedative Cement, Vitec, Cavitec, Caviline, Pulpotrex, Hydroxyline, Chembar, calcium hydroxide and water, and saline, were implanted in triplicate, in each animal, 3 rats per material, in the subcutaneous connective tissue of young Wistar rats. Triplicate specimens for histologic study were obtained after experimental periods of 2, 16, and 32 days. All of the materials implanted were initially irritating, except normal saline, which served as a control along with zinc oxide and eugenol, previously shown to be extremely mild. Six of the materials, Cavit, Serocalcium, Cargenol, Vitec, Caviline, and Cavitec initiated a severe inflammatory reaction, while Pulpotrex, Hydroxyline, Chembar, calcium hydroxide and water, and Gardinier Sedative Cement, produced a mild or moderate reaction. Although 6 of the pulp capping preparations contained calcium salts, only pure calcium hydroxide and water, Serocalcium Paste, and Hydroxyline caused the appearance of osteoid material or were responsible for calcification of the bundles of the panniculus carnosus muscle. The inflammatory reaction surrounding an implant, as well as its osteogenic influence, was independent of the pH of the materials. None of the materials containing zinc oxide and eugenol exhibited any osteogenic potential within the surrounding connective tissue. The results of this study warrant further investigation of possible correlations between the connective tissue reaction of the rat and reactions of the human dental pulp. The variety of responses to these different preparations points up the need for further testing of commercial (and other) preparations used for any purpose in dentistry.

192. Comparison of the Plasma Volume of Extracted and in Situ Teeth.—*Leon Kraintz and Charles W. Conroy, University of Texas Dental Branch, Houston.* The radioactivity of dog teeth, both freshly extracted and frozen in situ, was measured after systemic injection of radioactive iodinated serum albumin. The plasma volume was calculated by comparing the radioactivity of the teeth with that of a known volume of plasma obtained from the circulation. Teeth were extracted from the jaws of one side and the plasma volume determined. The contralateral jaws were surgically removed in toto and frozen in liquid nitrogen. The teeth were dissected from the jaws in the frozen state, and the plasma volume of the teeth was determined. Comparable values were observed between the plasma volumes of teeth freshly extracted and teeth frozen in situ in the same dog. Differences in tooth plasma volumes in different dogs were due to variations in pulp volumes. Deviations between the plasma volume values of freshly extracted contralateral teeth were not significantly different from deviations between the plasma volume values of

freshly extracted and contralateral frozen in situ teeth. It appears, therefore, that satisfactory measurements of plasma volume of the teeth of dogs may be accomplished by utilizing freshly extracted teeth.

193. EFFECTS OF ULTRASONIC APPLICATIONS ON DECIDUOUS AND DEVELOPING MONKEY TEETH.—*John W. Wakely, School of Dentistry, University of Minnesota, Minneapolis.* In a previous study (Northwest Dentistry, September, 1957) ultrasonic applications were made for 30 seconds with light and heavy pressure on the incisal and occlusal surfaces of deciduous monkey teeth and these animals were sacrificed at 2 days, 2 weeks, and 1 month. Where heavy pressure had been used, at the end of a month, general tissue damage was demonstrated on the operated tooth, its periapical area and the permanent tooth bud. Odontoblasts were all normal, but a moderate pulpitis was observed in the incisal and occlusal of the tooth buds, indicating that study of longer duration was necessary to ascertain if complete healing would take place, or if any permanent damage had occurred. In this study, the teeth of 8 Rhesus monkeys (age 10 months) were operated on with the ultrasonic instrument. Recommended pressure and clinically possible heavy pressure (5 to 6 pounds) were used for periods of 15 seconds in some quadrants and 30 seconds in other quadrants. The animals in this study were sacrificed at 3 months and 6 months. The gross appearance of the teeth and supporting structures were observed periodically. On the second postoperative day, a generalized swelling, necrosis and sluffing of the gingival tissue were observed. At 3 weeks, months earlier than these teeth would normally shed, most of the deciduous teeth had exfoliated. A complete denuding of the alveolar bone in all of the cases was observed where heavy pressure had been used, irrespective of the time variation (15 seconds or 30 seconds). At the date of sacrifice (3 months), in 2 of the cases there was sequestrum of the maxilla, and in another case a pathologic fracture of the mandible was observed. In the remaining cases, at 6 months postoperative, all of the deciduous teeth had exfoliated and some of the permanent teeth had also been lost.

194. PULP LABILITY AND REPAIR. II. THERMOGENESIS IN ULTRA-HIGH SPEED TECHNICS.*—*Leo Zach, Murry and Leonie Guggenheim Foundation, Institute for Dental Research, and New York University College of Dentistry, New York, and Gerson Cohen, VA Hospital, New York.* Patterns of pulpal response to dental lesions, trauma, medicaments, and filling materials were studied. Introduction of ultra-high speed operative instruments requires a critical, comparative evaluation of their effects on tooth structure. These data, in turn, require a more precise determination of the physical forces imparted to the teeth, with particular emphasis on heat generation and rotational velocity. Intrapulpal application of microthermistors, embedded in 22-gauge needles with leads to a direct reading, multichannel telethermometer permitted accurate determination of thermogenesis in operative procedures and a direct correlation with observed pulpal changes. Rotational speed of the low-torque operative devices was measured by a pulse meter, using as transducer a par-focal light source and selenium cell trained on a cutting disc which had been enamelled half white and half black. Intrapulpal temperatures in Rhesus monkeys ranged from 88° to 98° F. Air-turbine drilling runs using No. 557 carbide burs resulted, generally, in temperature drops of 8° to 10° F. in 30 seconds with a usual rise of 2° to 4° F. after the thirty-fifth second. After 40 seconds of

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drilling, using a carbide bur at 6,000 r.p.m. with no coolant, intrapulpal temperature rises up to 30° F. were recorded. Reproduction of various modes of cavity preparation were, after analysis for thermogenesis, repeated on the opposite homologous tooth for histopathologic assay. Two hundred and sixteen such preparations reveal a heightened proliferative response with bulbous masses of secondary dentin under ultra-high speed prepared cavities, considerable blistering as evidenced by microvacuolization under low-speed dry drilling, and a completion of pulpal reparative response generally within 30 days.

195. (Withdrawn)

196. Pulp Response to Heat.—*Harold H. Postle, William Lefkowitz, and Duncan McConnell, College of Dentistry, The Ohio State University, Columbus.* The pulp response to heat was tested by applying known temperatures to the surfaces of dog teeth. No cavity was prepared. The temperatures applied were 215°, 395°, and 900° F. Specimens were taken at 1 day, 1 week, and 1 month. A total of 88 teeth was equally divided among the various categories. The thickness of dentin and enamel was 2 mm. ± 0.5. Teeth subjected to 215° and 395° F. ran similar courses, the former being minimal. Ectopic odontoblasts and small areas of vesiculation were evident in 24 hours. After 1 week, inflammatory cells were present. The 1-month specimens showed complete recovery. The 395° F. specimens formed secondary dentin. The 900° F. series resulted in severe burn lesions. No ectopic odontoblasts occurred in areas of necrotic tissue. After 1 week, a marked hyperemia and inflammation were evident. The 1-month specimens showed complete recovery with new odontoblast formation, and reparative dentin. Two specimens showed localized pulp abscesses. Only one pulp was necrotic. The heat injury followed the isothermic lines, as did the formation of reparative dentin.

XXI. Lathyrism and Clinical Experimental Studies. March 22, Afternoon, Section A.

197. Tissue Mucopolysaccharides in Lathyric Rats.—*David J. Smith, New York State Dept. of Health, Albany, N. Y.* Previous reports from other laboratories have suggested, mainly on the basis of histochemical evidence, that there is a quantitative change in the level of mucopolysaccharides in the bones of lathyric rats. Because of the recognized limitations of histochemical determination of tissue mucopolysaccharides, a biochemical investigation of lathyric tissue was undertaken. Weanling rats received a ground commercial ration supplemented with 0.2 per cent aminoacetonitrille hydrochloride. After a suitable period, these animals and normal littermates were sacrificed and acetone powders of the long bones were preparad. Hexosamine determinations of such preparations disclosed no difference on a dry weight basis between normal and test groups. Precipitates prepared from alkali and neutral phosphate extracts of these bones showed no difference in hexosamine content, but larger amounts of material were obtained from lathyric bone. The viscocity of extracts from lathyric bones was always visibly greater than those obtained from the same amount of normal bone. These results indicate that the total amount of mucopolysaccharides remained unchanged in lathyrism, and that the lesion involved another soluble tissue component.

198. A Histologic Study of the Reaction to Injury in Lathyritic Rats.—*R. R. Bruns and George Krikos, The University of Rochester School of Medicine and Dentistry, Rochester.* The purpose of this experiment was to

ascertain how lathyritic rats respond to injury. Weanling male rats were divided into 4 groups of 16 animals each. Half of the animals in each group were made lathyritic by the addition of 0.4 per cent beta-aminopropionitrile to their diet of Purina Fox Chow. The rats in the other half of each group were pair-fed with Fox Chow and served as controls. After 9 days on this regimen, half of the control and half of the experimental rats were injured by dry heat applied to the shaved posterior thoracic wall. Four and five-tenths large calories per square centimeter was delivered in 1 second to a circular area 1 cm. in diameter. The remainder of the rats were injured by the subcutaneous injection of 0.2 ml. of 3 per cent Croton oil in olive oil. At 1 day and 5 days after injury, the animals were killed, the injured tissue excised, fixed in formalin, sectioned, and stained with hematoxylin and eosin. The sections of burned tissue from control rats at 1 day after injury showed a dense infiltration of inflammatory cells and abundant serous exudate. The sections from the experimental group showed very little cellular or serous exudate and very little disruption of normal tissue structure. At 5 days, the control sections showed dense accumulations of inflammatory cells walling off the injured tissue, while the experimental sections showed a very slight inflammatory reaction and very little disruption of tissue structure. The Croton oil sections of experimental and control animals were not significantly different.

199. THE EFFECTS OF AMINOACETONITRILE ON THE CHICK EMBRYO MANDIBLE.—*S. Jerome Zackin and Paul Goldhaber, Harvard School of Dental Medicine, Boston.* Although the mode of action of lathyrogenic drugs is unknown, recent reports have suggested a generalized disturbance of mesenchymal tissues. Various effects have been reported in different species. As part of the syndrome in the rat, a reduction in growth of the mandible has been reported. In the chick embryo, however, only a bending of the tibia has been noted with beta-aminoproprionitrile. Because the chick embryo offered a convenient, rapidly growing and differentiating site, another, more potent lathyrogenic agent, aminoacetonitrile hydrogen sulfate (Abbott), was tested in the present study. It was found that 1.0 mg. AAN injected into the yolk sac on the sixth day of incubation was sufficient to produce hypogenesis of the mandible or twisting of the beak, in addition to deformities of the tibia, in *every* embryo surviving to the thirteenth day of incubation. The survival rate of the embryos for the experimental period was 55 per cent (41 of 75) in the experimental group, compared to 81 per cent (42 of 52) for saline-injected controls. Similar effects were noted when this dose of AAN was injected at any time prior to the ninth day of incubation. Injection of 0.6 mg. AAN into the yolk sac of the 6-day embryo caused a mandibular deformity in exactly half of the surviving embryos, while the survival rate was 81 per cent (50 of 62). Since similar teratogenic effects caused by heavy metals, hormones, and enzyme antagonists may be prevented by nicotinamide, attempts were made to counteract the effects of AAN with this agent. The latter study is still in progress.

200. OBSERVATIONS ON THE GROWTH OF THE MACACA RHESUS MONKEY SKULL.*—*George S. Kendrick and John A. Cameron, Baylor University College of Dentistry, Dallas.* The purpose of this investigation was to observe certain bony changes following surgical removal of parts of the mandible. Three of 7 operated animals thus far have been sacrificed. (1) One animal received a bilateral condylectomy. One condyle was replaced with the distal epiphyseal head of the metatarsal. This animal showed no asymmetry of the dental midline. Both coronoid processes extended well above the zygomatic arch. The

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mandibular stump formed a covering of dense fibrous connective tissue. The other side showed the metatarsal graft to be in place. No increased ramal height was noted on the metatarsal graft side. Epiphyseal growth did not manifest itself. (2) The second animal sacrificed had a 10 mm. by 2.5 mm. unilateral section removed from the posterior ascending ramus, from the condyle along the posterior border. This animal demonstrated increased ramal height on the operated side. The ramus was positioned more laterally from the midline than the unoperated side. (3) The third animal received a unilateral condylectomy followed by a homogenous distal head of the fifth metatarsal graft. Condylar growth was replaced by epiphyseal growth. Upon sacrificing the animal, it was noted that a fibrinoid-type union existed with no growth at the metaphysis. However, some interstitial growth of the articular cartilage was noted and the bony epiphysis was about 2 mm. longer than when grafted. Final interpretation and data on the remaining animals will be reported at a later date.

201. Effect on Bone of Ultrasonic Cutting Device and Rotary Burs.—*Thomas A. McFall, George M. Yamane, and George W. Burnett, Walter Reed Army Institute of Research, Washington, D. C.* Since it is possible that an ultrasonic instrument might be adapted for use in bone surgery, a comparative study was made of the healing of bone cut with such an instrument and rotary burs. In this procedure, the tibias of white rats were surgically exposed, and a uniform cut was made with a chisel attached to the ultrasonic instrument and with carbon steel burs rotating at 4,000 r.p.m., some cuts being made dry and others under flowing sterile saline. Roentgenograms were taken at the conclusion of operation and at 5-day intervals; all animals were sacrificed on the forty-second postoperative day and tissues were prepared for histologic examination. The ultrasonic instrument, modified for use as a chisel, cut bone very efficiently in comparison to rotary burs, and there was less apparent damage at the end of the cutting period with the ultrasonic instrument than with rotary burs. Based on roentgenographic evidence, however, healing of bone was slower in the tibias cut with the ultrasonic chisel than it was in the tibias cut with rotary burs. Under all circumstances, healing was more rapid when flowing saline covered the cutting instrument than when it did not. The histopathologic finding of the tibias, 42 days postoperatively, somewhat parallels the radiographic findings.

202. Hemophilia, Corticosteroids, and Oral Surgery.—*N. Trieger, J. J. McGovern, and B. M. Jacobson, Massachusetts General Hospital, Boston.* Between Jan. 1, 1955, and Jan. 1, 1959, 22 dental operations were performed on 12 patients with hemophilia (8 with antihemophilic-factor deficiency and 4 with plasma thromboplastin-component deficiency). Six of these patients served as their own controls, that is, comparable extractions were performed, with and without the administration of steroids (prednisone), in addition to the usual methods of management. All patients were subjected to a similar pre- and postoperative regimen. All received prophylactic penicillin, atropine, no food for the first day, and liquids thereafter. All extractions were done by the same surgeon, and all sockets were treated only with topical thrombin powder and Gelfoam, and closed with a nontraumatic 5-0 Dermalon suture. No dental splints were used and, where indicated, pressure with a gauze sponge was applied to the bleeding area. Fresh frozen plasma was administered immediately preoperatively and every 4 hours for the first day (2 to 3 c.c. per pound of body weight). Further blood and plasma were given only when indicated. Sutures were removed on the fifth to seventh postoperative day.

Parameters for evaluating postoperative response to dental extractions included (1) frequent direct and indirect observations of blood loss, (2) amount of fresh frozen plasma or fresh whole blood required, and (3) duration of hospital stay.

TABLE I. RESULTS

	OPERATIONS	AVERAGE AMT. TX W.B./o.p.	AVERAGE AMT. TX F.F.P./o.p.	AVERAGE NUMBER HOSPITAL DAYS
With steroids 1955-1959	14	235 c.c.	2,650 c.c.	5
Without steroids 1955-1959	8	1,375 c.c.	4,500 c.c.	10
No steroids 1933-1954	11	3,300 c.c.	—	17

The results indicate improvement in the management of patients with hemophilia subjected to dental surgery. While on steroids, these patients required less blood and plasma, were relatively free of postoperative hemorrhage, and left the hospital sooner. We feel these data reflect the action of corticosteroids on the vascular bed and not in the coagulation process itself.

203. SOME FACTORS WHICH INFLUENCE BACTEREMIAS FOLLOWING DENTAL SURGERY.—*Sol Haberman, Ben Vargas, C. K. Collings, and L. A. Polter, Baylor University College of Dentistry, Dallas.* The incidence of bacteremia following oral surgery was found to be 38 per cent after exodontia, 24 per cent after gingivectomy, and 9 per cent following dental "prophylaxis." The organisms most frequently found were *Staphylococcus aureus* and *Streptococcus viridans.* The incidence of bacteremia was reduced when broad-spectrum antibiotics were given before surgery. With the greater number of extractions and greater extent of gingivectomy, there was an increase in positive blood cultures. In the case of exodontia, the presence of infection increased the number of positive blood cultures. The blood culture results found after gingivectomy were not influenced by the extent of tooth mobility and the depth of the gingival sulci of the teeth involved. It was found, however, that bacteremias were more frequent following gingivectomy on lower quadrants than on upper quadrants. Serial sections of tissues removed by gingivectomy were stained by the usual Hematoxylin-eosin method, Brown and Brenn modification of the Gram strain, and the Gomori methenamine-silver nitrate technics. These sections showed that bacteria and fungus-like organisms had invaded the soft-tissue side of the periodontal pocket. Frequently, the areas invaded failed to show chronic inflammation and the organisms seemed to migrate to the deeper tissues by perivascular infiltration. The disturbance of these microorganisms by manipulation or surgery may be one of the more important causes of bacteremias that often follow oral therapy.

204. TRANSIENT BACTEREMIA FOLLOWING DENTAL EXTRACTIONS.—*J. E. Wells, M. E. Greer, and W. J. Carter, The University of Kansas City, School of Dentistry, and the Children's Mercy Hospital, Kansas City, Mo.* Nine children ranging in age from 5 to 12 years were studied for post-extraction bacteremia. The number of teeth extracted varied from 2 to 13. Blood samples were drawn from the internal jugular vein immediately before and after the dental extractions. Bacterial growth was identified according to morphology, staining characteristics, and the degree of hemolysis on blood agar plates and in

trypticase soy broth. None of the preoperative blood samples were found to be positive and only two of the postoperative cultures were positive. The results were subjected to the single binomial test. It appeared that under the conditions of this experiment there is little justification for the assumption that deciduous teeth extractions are associated with bacteremia.

XXII. Salivary Glands and Saliva.
March 22, Afternoon, Section B.

205. Experimental Enlargement of the Submandibular Salivary Glands of the Rat.—*Herbert Wells and Paul L. Munson, Harvard School of Dental Medicine, and Harvard Medical School, Boston.* As reported previously, repeated amputation of the lower incisor teeth of rats is followed by a marked increase in the weight of the submandibular salivary glands. As part of a precise analysis of the whole phenomenon, an attempt is now being made to define the stimulus more specifically. The adequacy of a single amputation, as well as the rapidity of the response, is apparent from the fact that a significant increase in the dry and wet weight of the submandibular glands occurred within 24 hours after the initial amputation. In chronic experiments lasting 15 days, the greater the frequency of amputation the greater was the increase in weight of the salivary glands. The importance of continuous stimulation was also brought out by the rapid diminution in weight of the enlarged glands following cessation of a series of amputations. Periodic amputation of the tips of the lower incisors without exposure of pulpal tissue had no effect on the gland weight, indicating the necessity for direct stimulation of the pulp. This conclusion was strengthened by the observation that severe intra-oral ulcerated lesions, produced by daily application of a high frequency, coagulating electric current to the mucosa of the labial sulcus, also had no effect on the weight of the submandibular glands. Our data support the hypothesis that there are certain, as yet unidentified, receptors within the pulp tissue which, when stimulated by traumatic exposure of the pulp, initiate a chain of events, primarily neural, that leads to enlargement of the submandibular glands.

206. Occurence of Multinucleated Giant Cells in Cultures of Mouse Salivary Gland.—*Nicholas G. Grand and Lily Wang, University of Illinois College of Dentistry, Chicago.* The formation of foreign body giant cells has been a subject of many investigations. In the present study, mouse salivary gland tissue explants were embedded in fowl plasma and mouse embryo extract. Fibroblasts and epithelium-like cells appeared at the periphery of the tissue explants after 24 hours of incubation at 37° C. When the tissue cultures were kept in the incubator for at least 10 days, binucleated and multinucleated giant cells appeared at the periphery and migrated to as far as the fibroblasts and epithelium-like cells had extended. These giant cells varied in shape and size and invariably contained within their cytoplasm a large eosinophilic granular mass located in the center of each cell. Most nuclei of the giant cells which had this cytoplasmic mass, occupied an eccentric position. The giant cells contained between 2 to 8 nuclei which varied in size within the same cell from unusually small to twice the size of a nucleus of a macrophage. Each nucleus possessed a well-defined nuclear membrane with one or more nucleoli. On occasion, a nucleus would be constricted, suggesting amitotic division. We are of the opinion that these giant cells originated from macrophages that resided within the connective tissue stroma and proliferated and migrated from the explant. They might have been the result of a combination

of incomplete cell division and fusion. The stimulus for the formation and migration of these giant cells into the medium might have been the stasis of salivary secretions in the cultures.

207. PRELIMINARY OBSERVATIONS ON "INTRACELLULAR CANALICULI" IN SALIVARY GLANDS OF MICE.—*S. Y. Wang, University of Illinois College of Dentistry, Chicago.* Submaxillary salivary gland tissue from C-strain mice was removed and immediately fixed for 45 min. to 1 hr. in 1 per cent osmium tetroxide adjusted with veronal acetate buffer to a pH of 7.2 to 7.6. The tissues were washed and dehydrated according to the method of Palade prior to embedding in butyl and methyl methacrylate (9:1) with benzoyl peroxide as the catalyst. Sections approximately 0.025 μ thick were cut with glass knives on a Porter-Blum microtome and examined under a RCA electromicroscope (model No. EMU-2). Thicker sections (2 to 3 μ) were examined under the light microscope. Acini and ductal cells contained "intracellular canaliculi or double membrane"-like structures, located between the nucleus and the cell membrane in the basal part of the cell. In other acini, "ergastoplasmic" structures occupied the corresponding portion of the cell. Many mitochondria were present inside and/or between the "canaliculi or double membranes." Some of the "canaliculi" structures did not reach the periphery of the cell. This might have been due to an artifact occurring in the preparation of the section, or it might represent a stage of growth of these structures toward the periphery. The spaces in or between the "canaliculi" of some acini and ductal cells seemed dilated and filled with fluid. The central ends of some dilated spaces opened up toward the center of the acini or ducts. Whether or not these "canaliculi or double membrane"-like structures are associated with the intra- or extracellular spaces is not known.

208. ELECTROLYTE CONTENT OF RAT SALIVARY SECRETIONS.—*L. H. Schneyer and C. A. Schneyer, University of Alabama School of Dentistry, Birmingham.* Electrolyte patterns differ in rat submaxillary-sublingual and parotid salivas. Stimulation of submaxillary-sublingual gland by pilocarpine resulted in a saliva containing more potassium than sodium but which was distinctly hypotonic to serum. By contrast, saliva from stimulated parotid gland was richer in sodium than in potassium and was essentially isotonic to serum. Saliva was collected either directly from the duct or from the oral cavity following removal of one gland-pair. Findings obtained by either method were essentially similar and were consistent with previously observed differences in gland electrolyte patterns.

209. A COMPARISON OF THE MICROBIAL FLORA OF THE DENTAL PLAQUE AND SALIVA.—*K. O. Vowles, G. Bailey, M. A. Mazzarella, and L. S. Fosdick, Naval Dental Research Facility, Great Lakes, and Northwestern University Dental School, Chicago.* A sample of dental plaque was removed from each of the four quadrants of the mouth. Each sample was diluted with 0.7 ml. of water and homogenized. An aliquot was taken from microbial counts, and another aliquot was oxidized with dichromate for the determination of organic solids. A sample of saliva was also taken for microbial counts. The number of staphylococci, *Streptococcus salivarius,* yeast and lactobacillus was then determined in each plaque and saliva sample by means of selective media. The weight of plaque samples was determined by a calibration of the actual weight of plaques against the amount of dichromate reduced. On this basis, the number of the above microorganisms per unit of plaque was calculated and compared with the microbial population of the saliva. It was found that a close correlation generally existed between the salivary and plaque microbial counts.

210. Inorganic Constituents of Parotid Secretion.—*Thomas B. Weber, Virginia J. Lux, Naval Dental Research Facility, Great Lakes, Ill., and Myra Zimmerman, Evanston Department of Health, Evanston, Ill.* Previous studies at this laboratory showed quantitative differences between certain cations found in the parotid secretion of caries-immune and caries-rampant individuals. To formulate a comprehensive indagation of the inorganic materials present, anion determinations were conducted. Bilateral parotid secretion from 15 caries-immune and 26 caries-rampant naval recruits, averaging 19 years in age, was collected one or more times. The gland was activated by the chewing of flavored chicle gum intermittently on each side, for a total stimulated time of one hour. Aliquats of each sample or pooled samples were analyzed. The following anions were determined: bicarbonate, carbonate, fluoride, thiocyanate, nitrate, phosphate, sulfate, and chloride. Significant variations were found only in the latter three components. Inorganic phosphate was higher in the caries-immune group, while sulfate and chloride were greater in the caries-rampant group. For all anions, there was considerable overlapping between the two groups. The flow rate of the parotid secretion affected the quantity of some anions excreted, and occasionally a given material could not be detected.

211. Use of Eriochrome Blue S. E. (Mordant Blue 13, C. I. 16680) for the Determination of Calcium in Saliva.—*Leo M. Sreebny, Barbara B. Wanamaker, and Donald N. Bechlem, University of Washington, Seattle.* A method for the analysis of calcium has been reported by Yangisawa (1955) and modified by C. Rich (Rockefeller Institute for Medical Research) in which a blue-purple dye called Eriochrome Blue S.E. (the disodium salt of 3-[5-chloro-2-hydroxyphenylazo]-4, 5-dihydroxynaphthalene-2, 7-disulfonic acid) is used as a metal-sensitive indicator. The solution color is blue at and above pH 10. At this pH, red-colored metal complexes are formed. In strong alkaline solutions, Mg forms $Mg(OH)_2$, thereby releasing the reagent particles to attach themselves to Ca ions present in solution. The solution is titrated with EDTA. Since the end point between the metal-indicator complex and the free indicator is difficult to visualize, the titration is done directly in a Coleman Spectrophotometer at 610 mμ. The Eriochrome Blue S.E. method was applied directly to stimulated saliva (direct method) or to saliva in which calcium was first precipitated by oxalate (indirect method). Undigested and sulfuric acid digested controls were used in both the direct and indirect methods. These were compared with the standard Kramer-Tisdall method. The results showed that the direct Eriochrome Blue S.E. method on undigested saliva is (1) simple, (2) rapid (12 minutes), (3) accurate (duplicates differed maximally by 3 per cent; determinations agreed extremely well with the Kramer-Tisdall method), (4) the color is stable, (5) magnesium does not interfere, and (6) it is inexpensive.

212. Oral Dehydrogenases Coupled to Tetrazolium Salts.—*V. F. Lisanti, Tufts University School of Dental Medicine, and F. J. Lionetti, Boston University School of Medicine, Boston.* Whole human saliva was found to reduce tetrazolium salts to formazans which were read spectrophotometrically. Neotetrazolium chloride was one of the dyes tested. The optimal conditions for this assay were established as 2.0 ml. of the whole saliva, and 2.0 ml. of 0.11 M phosphate buffer (pH 8.1) containing 1 mM of sodium succinate and 0.8 mg. of neotetrazolium chloride. After incubation for 2 hours at 37° C., the tubes were centrifuged, the unreacted dye reduced with a small crystal of $Na_2S_2O_4 \cdot 2H_2O$, the purple supernatant decanted, diluted quantitatively five

times, and the absorbance read at 540 mμ. Controls contained all components without incubation. Reducing activity increased linearly with the volume of saliva and the concentration of dye. Reproducibility as shown by the standard deviation of the mean of ten determinations was ± 6.6. per cent. Reduction appeared to be largely confined to the bacterial sediment from which an active cell-free preparation of enzymes could be prepared by grinding (1.4 ml. of sediment obtained from 7.0 ml. of whole saliva with 5.6 ml. of 0.015 M. NaCl) with alundum in a ground glass homogenizer. The endogenous reduction in the preparation was negligible. The enzymatic nature of the reduction of dye was confirmed by heat inactivation and by the use of inhibitors. Succinate was the most effective substrate, while lactate, glucose-6-phosphate, citrate, and alpha ketoglutarate, alone or in combination with DPN or cyanide, did not affect the reaction. The bacterial origin of the enzyme in saliva was observed by microscopic examination of salivary components containing granules of purple formazan. The diurnal activity of fresh, unstimulated, whole saliva taken at hourly intervals correlated well with the changes in the flora.

XXIII. Clinical Studies. March 22, Afternoon, Section C.

213. The Importance of Equal or Proportionate Sample Sizes in Experiments.—*Neal W. Chilton and John W. Fertig, Columbia University Faculty of Medicine, New York.* In performing comparative clinical studies, it is advantageous to obtain equal numbers of readings. When this is not done, the analysis of the data becomes extremely complicated. In a clinical study of the retention on oral mucous membranes of two formulations of an adhesive agent on 9 subjects, different numbers of readings were made for each preparation and each patient. There are several ways of analyzing these data: trimming down the number of readings, or by random selection. The readings in each cell are then replaced by an average reading, and either a before-and-after "t" test, or a two-way analysis of variance, is performed. Analysis of variance can also be performed on these trimmed data directly for additional testing, but these methods do not utilize all the data. Another approach would be to trim the data down to proportionality. The simplest way would be to equalize the number of readings for each preparation for each individual. The analysis of variance can then be performed, but it is somewhat more complicated, and some of the original data are not utilized. To utilize all the data in the analysis, complicated weighting procedures are employed, in which the difference between the average cell readings for each individual is weighted by the reciprocal of the variance of this difference. The analysis of variance is much more complicated and the F tests cannot be performed according to Yate's method, which is applicable to completely fixed models only.

214. Pharmacologic Studies on Several New Local Anesthetics.—*George D. Wessinger, F. P. Luduena, and James O. Hoppe, Sterling-Winthrop Research Institute, Rensselaer, N. Y.* Several new local anesthetic compounds were synthesized and studied in the laboratory. The potency was estimated by determining the threshold anesthetic concentration in guinea pigs. The acute toxicity was determined intravenously in mice. Local irritation was estimated by the trypan blue test in rabbits. The results obtained in terms of procaine ratios are shown in the following table.

The addition of a vasoconstrictor resulted in increased potency and longer duration in the usual way.

COMPOUND	POTENCY (PROCAINE = 1)	TOXICITY (PROCAINE = 1)	IRRITATION (PROCAINE = 1)
Win. 14,941	3.7	2.3	1.3
Win 16,171-2	2.1	2.5	1.2
Win 16,194-2	6.6	6.0	2.9

215. The Cytotoxic Effect of Local Dental Anesthetics on Tissue Culture.—*Doran D. Zinner, James M. Jablon, Murray Sanders, and Milton S. Saslaw, National Children's Cardiac Hospital and University of Miami, Miami, Fla.* The frequent use of local anesthetics in dental procedures emphasizes the need for study of possible cytotoxic effect. Tissue culture technics, using monkey kidney and HeLa cells, were adapted to estimate degrees of toxicity. Nine commercial anesthetics were tested: (1) propoxycaine HCl + procaine HCl + levonordefrin; (2) propoxycaine HCl + procaine HCl + levarterenol; (3) procaine HCl (2 per cent) + tetracaine HCl (0.15 per cent) + phenylephrine HCl (1-25,000); (4) procaine HCl (4 per cent) + phenylephrine HCl (1-25,000); (5) metabutoxycaine HCl (1.5 per cent) + epinephrine (1-60,000); (6) metabutethamine HCl (3.8 per cent) + epinephrine (1-60,000); (7) Lidocaine HCl (2 per cent) + epinephrine (1-100,000); (8) Lidocaine HCl (2 per cent) + epinephrine (1-50,000); (9) Lidocaine HCl (2 per cent). All contained buffers, antioxidants, and/or preservatives. Tissue cells were grown in monolayers in test tubes. Anesthetics, concentrated and in 1:10 dilution, were added to cultures and incubated at 37° C. Contact times of anesthetics with cell cultures were 3, 6, or 24 hours. After each period, cellular destruction was estimated by direct microscopic examination and by viability stains. The condition of cells was checked further by ability to metabolize in the presence of fresh nutrient media. After each period, anesthetics were removed, cultures were rinsed thoroughly with balanced salt solution, and fresh nutrient was added. Ability to metabolize was evident by pH changes after 1 to 3 days. After 3 hours' contact all undiluted anesthetics were toxic. Anesthetics (1:10 dilution) showed varying degrees of toxicity at the end of each period, although none was completely toxic after 3 hours' contact. Greatest toxicity changes were apparent after 6 and 24 hours' contact; some anesthetics being more toxic than others. Results were similar, when monkey kidney or HeLa cells were used, at the end of 3 and 6 hours' contact. After 24 hours' contact, however, HeLa cells showed greater destruction than monkey kidney cells.

216. Destruction of Local Anesthetics in Blood From Human Beings.—*J. Roy Doty, Edward G. Feldmann, and Henry M. Koehler, Council on Dental Therapeutics, Division of Chemistry, American Dental Association, Chicago.* In vitro studies were made of the rates of enzymatic degradation of several of the compounds which are employed in dentistry as local anesthetic agents. The procedures were designed to simulate the normal physiologic environment with respect to pH, temperature, concentration of blood constituents, and other factors. This was accomplished in part by the addition of microvolumes of buffered substrate (anesthetic compound) solution to larger volumes of serum and whole blood. The anticipated inverse relationship was noted between the in vitro rate of destruction and the clinically observed duration of anesthesia. The data were taken from continuing studies of the rate and nature of the detoxification mechanisms for injected local anesthetics.

217. Adaptation of Amalgam to Cavity Walls Tested With Ca^{45}. *A. T. Hampel, School of Dentistry, University of Minnesota, Minneapolis.* The adaptation of silver amalgam to cavity wall surfaces produced with different cutting

instruments was tested with Ca^{45}. Five different instruments (rotary instruments at 15,000 r.p.m.) served as the variables. All other factors were kept constant within experimental error. Autoradiographs revealed leakage between the cavity walls and the restorative material in all five groups, but in varying degree. Numerical values were obtained by using a microdensitometer for the evaluation of the autoradiographs. The order of decreasing amounts of penetration was (1) 557 cross-cut carbide bur, (2) garnet medium sandpaper disc, (3) 557 cross-cut steel bur, (4) diamond 180 mesh, and (5) chisel. The most desirable surface appeared to be the one produced by the chisel. The least desirable surface seemed to be produced by the cross-cut carbide bur.

218. MICROLEAKAGE OF GOLD FOIL FILLINGS.—*James B. Taylor, Ellery C. Stowell, Jr., John F. Murphy, and W. W. Wainwright, VA Hospital, Long Beach, and 4168 Charlene Drive, Los Angeles, Calif.* Studies in this laboratory using radioisotopes have shown microleakage in virtually 100 per cent of the amalgam fillings tested. This finding is in qualitative, and often in quantitative, agreement with similar studies in other laboratories, but conflicts with most results where other test substances, such as dyes, have been used. Since the isotopic technic appears to be the most rigorous test of marginal adaptation known, it was of interest to investigate microleakage in gold foil fillings by this technic. In a series of 28 gold foil fillings some microleakage was found in every case. Nevertheless, a striking difference from the results with amalgam fillings was obvious. With one exception, penetration in the gold foil fillings was limited to the filling or to the margin itself, with no penetration into the underlying dentin and pulp. Penetration was frequently limited to the enamel in Class V foil fillings. Another contrast to amalgam fillings was the frequent finding of isotope in low concentration throughout the body of the foil filling. In a number of such cases no marginal leakage per se could be detected. In agreement with clinical experience, the conclusion seems justified that gold foil fillings are a better clinical risk than amalgam fillings for reducing the incidence of secondary caries or other internal changes subsequent to the initial caries process.

219. MICROLEAKAGE OF IN VITRO AMALGAM FILLINGS TO I^{131}-LABELED HUMAN SERUM ALBUMIN AND NaI^{131}.—*W. W. Wainwright, Ellery C. Stowell, Jr., and James B. Taylor, 6148 Charlene Drive, Los Angeles, and VA Hospital, Long Beach, Calif.* Microleakage of Class V in vitro amalgam fillings was less extensive when I^{131}-labeled human serum albumin was used as the tracer than when iodine itself was used. Nevertheless, clear-cut evidence of microleakage was found in 24 of the 25 fillings tested witih labeled albumin after a 30-minute immersion period. Penetration into intact dentin beneath freshly cut unfilled cavities revealed a similar difference between albumin and iodide. The implications of these findings were discussed.

220. (Withdrawn.)

XXIV. ABSTRACTS OF PAPERS PRESENTED TO THE DENTAL MATERIALS GROUP*

M1. A METHOD FOR ANALYZING STRESSES IN AMALGAM RESTORATIONS.—*Louis G. Terkla and David B. Mahler, University of Oregon Dental School, Portland.* The "Brittle Model" method for analyzing stresses in engineering structures is characterized by its ready application to the three dimensional problem, whereas photoelastic technics suffer in this regard. It was the purpose

*This meeting coincided with that of the International Association for Dental Research.

of this investigation to evaluate this method of stress analysis as to its application to the problem of the design of silver amalgam restorations. Plaster of Paris is the material used for the restoration, since, under proper conditions of test, it exhibits a straight-line stress-strain relationship to the fracture point. Since amalgam under masticatory stress exhibits brittle characteristics, this method appears significantly applicable. Tooth models with prepared cavities eight times actual size were made of improved dental stone and the restoration made of dental plaster. Force was applied to the restoration and the force to cause fracture was recorded. Preliminary results with this method, when compared to fracture results of amalgam packed into Ticonium teeth (actual size), indicate a similar plane of fracture for both amalgam and plaster, and improved reproducibility of fracture load for the plaster restorations.

M2. An Evaluation of the Eames Amalgam Condensation Technic.—*E. W. Skinner, and G. T. Mizera, Northwestern University Dental School, Chicago.* Mercury-alloy ratios = 1 are used in the Eames amalgam condensation technic with essentially the usual technic of trituration and condensation. It has been demonstrated that an increase in one-hour compressive strength can be obtained with the Eames technic, which may be as much as three times the value obtained with the usual method. This increase in strength appeared to be more marked with the larger alloy particle sizes, provided the alloy contained zinc. No significant differences in dimensional change, flow or 7-day compressive strength were found when compared with the similar properties obtained with the usual technic.

M3. Junctions Between Set Amalgam and Added Amalgam.—*Herbert D. Ayers, Jr., Columbia University, New York City.* The placement of new amalgam restorations in contact with previously placed amalgam fillings has been accomplished clinically in many cases. The junction between a set and an added amalgam has been the subject of this study. A test filling was placed in a plastic block. Twenty-four hours or more later, a recess was prepared to receive an addition of new amalgam. Two methods of removing the debris were used. The cavity was filled with a new mix of amalgam. The setting of the amalgam at the junction with the old was studied microscopically as it proceeded. The mercury solution was observed as solution of the alloy and crystallization of the mercury silver and mercury tin compounds proceeded. After 24 hours or more, the surface of the junction was polished and etched. Photographs showed the nature of the junction between the two mixes of amalgam. Areas of structural continuity were shown, as well as areas of distinct demarcation.

M4. Setting Time of Dental Amalgam as Influenced by Heat Treatment.—*Lloyd Baum and Burton Everett, School of Dentistry, College of Medical Evangelists, Loma Linda.* This study, which involves the manufacturing process of amalgam alloy, was directed toward the setting time of amalgam as influenced by heat treatment of alloy particles for various periods of time at temperatures above 100° C. A non-zinc alloy was melted, cast into an ingot, and machined in contact with dry ice to 325 mesh particle size. Alloy samples were heated for 5 minutes to 15 hours at temperatures varying from 100° C. to 250° C. Setting time of amalgam prepared from the above samples varied from 5 to 30 minutes. Setting time was measured by a scratch test. Amalgamated specimens were packed into a broad flat mold. Periodically, every 30 seconds, a 90 degree angle carving blade weighing 33.6 grams was drawn across the setting amalgam, producing V shaped scratches which were measured for

width with a microscope and a filar eyepiece. Setting amalgam which produced a 0.50 mm. width groove was arbitrarily chosen as comparable in consistency to the amalgam which makes clinical carving difficult. Findings of this study revealed a marked increase in setting time of amalgam made from alloy which had been subjected to temperatures over 100° C. for even short periods of time. For best clinical results, this phase of the manufacturing process should be carefully controlled. Although several interesting observations were made, no comprehensive study was conducted pertaining to mercury content, amalgamation time, crushing strength, or dimensional change.

M5. DETAIL DUPLICATION TEST USED TO EVALUATE ELASTIC IMPRESSION MATERIALS.—*Alex Dell, Herbert D. Ayers, Jr., Columbia University, New York, Richard Henry, and Ralph W. Phillips, Indiana University, Indianapolis.* The quality of surface-detail duplication by various impression material, model material combinations was studied. A scale of seven graded sized indentations was used as a test pattern. A Knoop indenter applied with seven different loads formed the scale on a stainless steel surface. The indentations ranged from 34 to 422 μ. Ten elastic impression materials were used. Eighty-one scales were impressioned with each material. When the material had set it was removed. The indentations duplicated were counted. The ability of the impression material to impart indentations to a gypsum product model material was tested by pouring the model material against the impression after counting the indentations and within 10 minutes after separation. The conclusions follow. (1) Thiokol rubber and Siloxane impression materials appear superior to other types of impression materials tested in ability to reproduce surface details. (2) The gypsum product model materials tested reproduced fewer details than the elastic impression materials registered. (3) Thiokol rubber and Siloxane materials impressioned more surface details that were duplicated on the models than reversible hydrocolloids or the alginates. (4) Alginate impression materials are inferior in ability to duplicate surface detail.

M6. SURFACE REPRODUCIBILITY OF ELASTIC IMPRESSION MATERIALS.—*Takao Fusayama and Hiroyasu Hosoda, Tokyo Medical and Dental University, Tokyo, Japan.* Accurate surface reproducibility is essential for indirect dental dies. Modifying W. A. C. Miller's technic, very fine lines, 8 to 40 μ wide, were cut on an original metal die surface, and their reproducibility on resultant stone dies was observed when impressed with alginate or Thiokol materials. Fixing in a 1 per cent $ZnSO_4$ solution for 30 seconds and pouring with a rather pure hydrocal-type stone (no densite) yielded the best result for all different alginate materials tested. With adequate manipulation and the best materials, the alginate impression could reproduce a line as fine as 10 μ on stone dies. Thiokol impression also could reproduce the 10 μ line, regardless of the type of poured stone, but failed to reproduce the 8 μ line, as alginate impression did.

M7. DIMENSIONAL STABILITY ANALYSIS OF SILICONE IMPRESSION MATERIALS.—*Melvin R. Lund and Thomas Thompson, College of Medical Evangelists, Loma Linda, Calif.* During the past few years, the success or failure of a significant portion of restorative and prosthetic dentistry has revolved about the accuracy of our elastic impression materials. Of most recent interest in the area has been the development of the silicones as an impression base. Several desirable features, such as the elasticity, ease of handling, and patient acceptance, were readily noticed, but it remains for a longer span of time to determine its acceptance from a dimensional point of view. This presentation represents a step

in determining the range of dimensional stability of silicones on the market at the present time. The test method utilized a steel bar which had machined into it a mathematically ground tapered slot. Into this slot was precisely fitted a tapered bar in such a manner as to be flush at the end and across the top surface. Special fenestrated trays were keyed to the slot in such a manner so as to have equal distribution of impression material. These impressions were poured in a densite stone to minimize setting changes. Upon separating the stone from the impression, the bar was placed into the stone slot. If no dimensional change is manifest, the bar will fit flush; if the impression is undersize the bar will protrude, and if oversize the bar will intrude into the slot. These deviations, if any, are measured by a comparator dial gauge, mathematically graduated. The time of pouring the impressions was varied in order to determine any dimensional change. Results showed that when the silicone impressions were poured within 30 minutes, the resulting accuracy was dependable, well within a range needed to answer clinical problems.

M8. The Clinical Effect of Warping a Denture.—*George C. Paffenbarger, American Dental Association Research Division, National Bureau of Standards, Washington, D. C., and Julian B. Woelfel, College of Dentistry, Ohio State University, Columbus.* A complete upper artificial denture, having a cross-linked acrylic resin base, was warped by boiling in water. This treatment resulted in a shrinkage of 0.4 mm. across the posterior of the denture without seriously affecting its fit and serviceability. Additional warpage induced by heating the denture, encased in gypsum, in boiling water, caused an additional shrinkage of 0.5 mm. across the posterior. The denture could still be worn, but its fit was affected adversely. The relationship was good between the fit of the denture on a gypsum cast of its tissue-bearing surface and changes in dimension of the denture. Both of the foregoing correlated well with the patterns given by coating a pressure-indicating paste on the tissue-bearing surface of the denture.

M9. Dimensional Accuracy of Denture Base Resins.—*Julian B. Woelfel, Ohio State University, Columbus, and George C. Paffenbarger and William T. Sweeney, National Bureau of Standards, Washington, D. C.* Dimensional changes occurring in both technic and complete artificial dentures were measured during processing and for a period of 12 months thereafter. Eleven resins were tested using the recommended technics for processing them. The resins were eight types of polymethyl methacrylate, one vinyl-acrylic copolymer, one polystyrene, and one epoxy. The processing technics used were (1) compression molding with and without trial packing, (2) directional hydraulic compression, (3) injection with both "dry" and moist" heat, (4) transfer molding, (5) high-temperature vacuum cure, and (6) the pouring method. Linear dimensional changes occurring from molar to molar and across the posterior borders were measured on 150 dentures. Mean linear curing shrinkage for these dentures was –0.5 per cent, or approximately 0.25 mm. The linear changes which occurred during processing were usually greater than subsequent changes. Most dentures obtained relative dimensional equilibrium after 3 months' storage in water or after 3 months' service in the mouth. Upper dentures changed slightly less than lowers; thick dentures less than thin ones (uppers and lowers). The epoxy resin had low curing shrinkage followed by a large expansion when stored in water or when in service. Twenty-two dentures examined 2 and 6 years after insertion exhibited minimal dimensional change, usually less than 0.3 mm. Clinical examination of 73 dentures made in this study failed to establish evidence of superiority of any specific processing technic or resin.

M10. EVALUATING DIMENSIONAL ACCURACY OF DENTURE BASES WITH A MODIFIED COMPARATOR.—*D. H. Anthony and F. A. Peyton, University of Michigan, Ann Arbor.* A method has been developed for comparing the inner contours of complete dentures with the contours of a master impression. In this technic, both the metal impression and the dentures are mounted separately in the same relative position on a comparator by means of a mounting guide and reference points located in a metal ring. Contours are reproduced in graph form from a series of measurements taken in a transverse direction and along the midline. The accuracy of fit at any point is determined by measuring the shortest distance between the contour lines. A series of heat and self-cured plastic dentures were processed under similar conditions and representative contour lines were established for comparing each with dentures made with other materials and technics. Both types appeared to contact the model in the flange areas of the upper dentures and to fit most accurately in the anterior portion of the lower dentures. The reduced shrinkage of the self-cured plastic allowed a further seating of the dentures to the model and a better over-all fit. Discrepancies in the center of the palate were approximately 1 to 2 thousandths of an inch, compared to 8 to 9 thousandths for those which were processed of heat-cured plastic. After 8 months' storage in water, the heat-cured plastic expanded and allowed the dentures to be seated somewhat further on the model. Clinically, the compressive force required to seat any of these dentures would appear to be within the tolerance limits of the oral soft tissues.

M11. AN EVALUATION OF THE AUTOPOLYMERIZING RESINS FOR DENTURE RELINES.—*J. A. Jansen, University of Malaya, Singapore.* Some autopolymerizing resins are recommended for direct relines at the chair. The method is supported in some quarters of the dental profession. Mucoperiosteal tissues often show adverse reactions when dentures are so relined. Three factors have been cited as responsible: the heat of reaction, excessive monomer and surface porosity. The study to evaluate the autopolymerizing resin for relining at the chair was based on how nearly it approached the ideal impression material. After polymerization, the material was expected to have the properties required of a denture base material. At the thickness used in relining, the heat of reaction exceeded the limit tolerable by the oral tissues (60° C.; 140° F.) only when accentuated by mouth temperature (37° C.; 98.6° F.). Moisture contamination did not seriously increase the heat. Flow tests showed the autopolymerizing resins to be inferior to an impression paste and laboratory-press pressure would be more ideal for conformation of the resins. Body-heat accelerated hardening, and unless sufficient pressure was exerted when seating the denture, increased vertical height could be expected. On removal from the mouth to cure in air, peripheral distortion occurred from contraction of the material and release of strain in the denture base. This would cause tissue ulceration, bone resorption, and loss of fit of the denture. Excess monomer was leached out in water. Surface porosity occurred. There was some yellowing of the resins on standing. It is suggested that autopolymerizing resins are not suitable for relines at the chair and a laboratory method is described.

M12. SILICONE RUBBER VULCANIZED IN A PLASTER MOLD.—*Eugene J. Molnar, Coe Laboratories, Inc., Chicago.* In many fields, application of this nontoxic elastomer with unusual heat-and-water-resisting properties is handicapped by use of metal molds to withstand processing temperature and high pressure. A low water-ratio, autoclaved plaster (Super-Cal) was used as a mold in a

heavy dental type flask. Mix was vacuum-treated before pouring. After hardening, free moisture was dried off to promote a smooth, gas-free surface. A direct wax pattern or an electroplated one (to transform plating to mold cavity) can be used. In the latter, perfectly finished specimens are repeatedly cast and easily removed. Using conventional rubber molding equipment, the freshened, catalyst-compounded silicone gum should be somewhat overpacked in a preheated flask, then closed on a continuously heated press (about 100° C.) and held under pressure for 30 to 45 minutes. Curing time greatly influences the physical properties of the rubber; the longer the time, the higher the durometer hardness and tear strength. Linear shrinkage is unusually low, about 0.5 per cent (G. E. Silicone, blend of SE 452 + SE 482). After cure, the cast was removed from flask and placed in a circulating air oven to eliminate uncross-linked molecules and by-products (benzoic acid, carbon dioxide, hydrogen, etc.). The simplicity, adequate strength, and minimum cost of this mold make it possible to process intricate and deeply imbedded objects; facial restorations, ears, teeth protectors, or flexible molds. Work is in progress to bond this rubber to acrylic and metal surfaces for denture relining purposes.

M13. Toxicity of Diethylene Triamine.—*William L. Kydd, School of Dentistry, University of Washington, Seattle.* Diethylene triamine was applied to the flexor surface of the forearm of 50 subjects. This was evaluated 24 to 48 hours later. Nine subjects had positive reactions. Six had + 1 reaction, and three had + 2 reaction. Forty-eight hours after application, 9 subjects had positive reactions, 2 subjects had + 1 reactions, 3 subjects had + 2 reactions, and 4 subjects had + 1 reactions. Fourteen days later diethylene triamine was applied to the original test sight on the same 50 subjects. There were eight reactions; six + 1, and two + 2 reactions. The 9 subjects who had positive reactions on initial application showed no reaction on reapplication.

M14. Air-Pressure Device for Determining Thickness of Small Areas of Undecalcified Tissue Sections.—*Barrie Gillings, Michael Buonocore, Eastman Dental Dispensary, Rochester,* and *Paul Pagerey, Taylor Instrument Co., Rochester.* A simply constructed, inexpensive air-pressure device for measuring accurately and rapidly the thickness of small areas of the surfaces of undecalcified tissue sections is described. Pressure readings are recorded when a section is placed between two opposing air streams emanating from tiny jets which are connected to duplicate air-pressure gauges. These pressure readings are converted to thickness in microns by means of calibration curves obtained, using an accurate micrometer screw gauge. Feeles gauges of known thickness are used as standards for setting the instrument to measure different ranges of section thickness. Measurements of standards indicated a precision of 10 parts per 1,000 and an accuracy within 2.5 per cent. With this technic, the sections are not subject to crushing, as with the use of a micrometer screw gauge. This reduces the possibility of damage to the sections and, moreover, measurements can be made on fragments as small as 1 mm. across.

M15. Adaptation of In Vivo Restorations as Assessed by Ca^{45}.—*Wm. H. Gilmore, M. L. Swartz, and R. W. Phillips, Indiana University School of Dentistry, Indianapolis.* Amalgam, silicate, zinc phosphate cement, resin, and zinc oxide and eugenol restorations were placed in the teeth of human beings and dogs. The effects of cavity liners were also studied with some of the materials. Marginal adaptation was evaluated by permeability to Ca^{45} ions and the degree of leakage recorded by autoradiographs. Time intervals of 48 hours, 30 days, and 6 months were used. Various degrees of leakage were exhibited by the different materials. Although considerable penetration occurred

initially at the margins of amalgam restorations, leakage seemed to diminish as the restoration aged. Leakage was also observed with silicate and zinc phosphate cement; however, there was considerable variation among individual specimens. The restorative resins, with or without the recommended cavity seal, were quite effective for the short time interval involved. The results in human teeth, extracted at 1 week and 1 month, were generally in agreement with those obtained in the animal portion of the study. There were some differences, however, in the degree of leakage observed with some materials.

M16. STUDY OF MARGINAL SEAL OF DENTAL RESTORATIONS USING C^{14} LABELED FRUCTOSE.—*J. C. Cantwell, K. R. Cantwell, C. K. Claycomb, and D. C. Gatewood, University of Oregon Dental School, Portland.* Previous studies designed to check the marginal seal of dental restorations have used radioisotopes of small molecule or atom size in the ionized state. In this study, the larger molecule, fructose, uniformly labeled with C^{14}, was used to determine if molecular size is an important factor. A simple four-walled cavity was prepared on the labial surface of extracted anterior teeth approximately halfway between the cervical line and the incisal edge. After the placement of the amalgam restorations, all specimens were stored in a humidor for at least 24 hours. The samples were then removed, polished, and completely waxed, except for a window extending for 1 mm. in all directions from the margins of the restoration. The window area was cleaned with acetone and placed into 50 ml. of an aqueous fructose solution containing 0.4 mc. of C^{14}. The samples were left in the solution for intervals of 6, 15, and 24 hours. The teeth were dried with tissue paper. After removal of the wax, the amalgam was ground to remove surface activity. This was done to avoid contamination while sectioning the sample. New Moyco separating discs were used for each specimen in preparing sagittal sections in the buccolingual plane. The sections were surface sanded and placed directly upon dental film emulsion. All film were lightly fogged with safety light for later orientation and exposed for 48 to 72 hours. The radioautographs exhibited a fine line of exposure around a majority of the amalgam restorations studied. Experiments are in progress to study marginal seals of other restorative materials.

M17. FLUORIDE UPTAKE OF ENAMEL FROM CERTAIN DENTAL MATERIALS.—*R. D. Norman, R. W. Phillips, and M. L. Swartz, Indiana University School of Dentistry, Indianapolis.* Previous studies on enamel have indicated a correlation between solubility and the fluoride content of the dental material. This investigation was a continuation of that research designed to determine the quantity of fluoride released by these materials and the resultant uptake by enamel. The materials used were Kryptex, FluorOn, silicate cement, zinc phosphate cement, a nonfluoride silicate cement, a fluoride-containing zinc phosphate cement, and two experimental resins containing 2 and 5 per cent sodium fluoride. The results of the dissolution of fluoride from the various materials indicate that only a very small amount of fluoride was dissolved out of the test specimens. This dissolution never exceeded 2 per cent of the fluoride present in the material. The dissolution of fluoride from the dental materials occurred over a relatively short period of time. Samples of pooled, powdered enamel were analyzed and found to contain an average of 119.5 ppm fluoride. This compares with previously reported data. As would be expected from enamel solubility studies, the largest amounts of fluoride uptake occurred with enamel samples in apposition with silicate cement and FluorOn resin. There was a 496 per cent increase for the silicate. The fluoride-containing zinc

phosphate cement, the nonfluoride-containing zinc phosphate cement, and the nonfluoride fluxed silicate cement resulted in a decrease in fluoride content of the enamel. A comparison of these data and the results on enamel solubility indicated a relationship between the release of fluoride by materials and the reduction in enamel solubility.

M18. The Addition of Silver Amalgam Alloy to Zinc Phosphate Cement.—*George K. Armen, Jr., and David B. Mahler, University of Oregon Dental School, Portland.* To evaluate the clinical significance of amalgam alloy addition to zinc phosphate cement, the following physical tests were employed: compressive strength, transverse strength, and solubility and disintegration. One typical zinc phosphate cement was used throughout this investigation and, where applicable, A.D.A. Specification Test No. 8 was utilized. Three consistencies of mix were evaluated with and without alloy added. These were designated as cementing mix, midpoint mix, and base mix. The results of compressive strength tests indicate that initially the addition of amalgam alloy reduced the strength of the cement, and only in amounts greater than that of the weight of the cement powder in the mix did the strength approach that of the nonalloy added mix. The transverse strength data (a measure of "edge strength") show that the addition of 3.0 grams of amalgam alloy, which was greater than the weight of cement powder, significantly increased the strength of each of the mixes tested. Solubility and disintegration were evaluated in distilled water and also in citric acid. The results suggest that there was little or no difference between the three mixes tested, but there was a reduction in disintegration when 3.0 grams of alloy particles were added as compared to no alloy added.

M19. A Study of the Behavior of Pattern Wax.—*George M. Hollenback, College of Medical Evangelists, Loma Linda, Calif.* A research project is under way, its objective being a complete re-evaluation of the casting process as used in the production of single tooth restorations. It will be appreciated that many technicalities, and also many difficulties, will be encountered in this research. The physical properties of each of the three materials used must be thoroughly evaluated. In view of the fact that the linear casting shrinkage of gold and its alloys has been, we believe, adequately determined, this material will not receive further consideration. The physical properties of pattern wax and investment materials will be evaluated. It seems logical that these materials, when acting in combination, will not be the same as properties established when each material is investigated separately. The method of approach in the study of wax is as follows: various types and sizes of wax patterns have been made, using a number of methods of wax manipulation. These patterns are first evaluated for the accuracy of adaptation and are then subjected to various environments and temperatures. An instrument has been designed, using a measuring microscope, which permits a dimensional study of each pattern at any desired time. Any distortion which may have occurred can also be evaluated. Other instruments have been designed for a study of the behavior of the wax after the pattern has been invested. These instruments permit an accurate determination of distortion of the pattern, should this occur. The results secured show, on the whole, that pattern wax, when correctly manipulated, is quite a stable material.

M20. The Dimensional Behavior of Wax Patterns Made to Gypsum Dies.—*David B. Mahler, and George E. Miller, University of Oregon Dental School, Portland.* The purpose of this investigation was to analyze technics in forming wax patterns so as to determine which procedures would produce

the most dimensionally accurate pattern. An MOD brass die with pyramidal indentations was reproduced in gypsum, and measurement of dimensional change was made to these indentations as they were reproduced in the gypsum die and the subsequently formed wax pattern. The greatest change occurred in the buccolingual direction where the wax was not restrained by the die. Under constant test conditions, soft waxes produced less change than hard waxes, and small additions of wax produced less change than large additions. A water swager, which maintained hydrostatic pressure on the pattern during cooling, resulted in the best reproducibility of die dimension by the wax pattern.

M21. TECHNICAL PROBLEMS IN THE MANUFACTURE OF DENTAL MATERIALS.—*W. F. Talbot, Kerr Mfg. Co., Detroit.* The critical requirements in respect to physical and chemical properties of dental materials impose demanding problems on the manufacturer. Manufacturing to a given formula does not produce uniformly satisfactory materials. Raw materials of high purity and uniformity, as measured by usual methods of control, frequently produce finished items demonstrating highly erratic results in respect to dental requirements. These facts compel in-process testing of each lot of material, together with adjustments in formulation to assure conformity with established standards. In addition to variations in raw material as received, such properties as flow and setting time are further influenced by age of materials, conditions of storage, climate and weather, and physical treatment during manufacture. Illustrations of technical problems in manufacturing will be drawn from such products as zinc oxide-eugenol pastes, waxes, and dental investments. Failure to solve these problems creates difficulty in dental use, and the types of difficulty will be pointed out. In the case of materials whose use involves chemical reaction, such as the synthetic rubber impression materials, these reactions are strongly influenced by conditions of usage. Even though carefully adjusted to meet certain specifications of setting time and time required to achieve satisfactory development of rubber characteristics, the results obtained under widely varying climatic conditions will show substantial differences. This situation is illustrated in the accompanying diagrams indicating the effect of temperature and humidity on setting and curing time of both Thiokol and silicone rubbers.

M22. AUTORADIOGRAPHS OF SOLDER JOINTS WITH FLUORINE^{18} (PART II).—*Saul M. Bien and Herbert D. Ayers, Jr., Columbia University, New York.* Further data are now available concerning the investigation of the nature of the bond in soldering rustless alloys of the nickel–chromium and cobalt–chromium types. Additional autoradiographs were made of 22 specimens. The presence of fluorine^{18} was shown at the interface between the solder and the rustless alloy wire in 21 specimens. Six controls of similar solder joints using no fluorine^{18} in the flux were negative. Ten specimens of gold alloy wires soldered with silver alloy, using flux containing fluorine^{18}, showed radiopacity in areas of inclusions in the body of the solder. Radiopacity was noted on the film in contact with various surfaces of the cobalt-chromium-molybdenum cast wire and the 18-8 austenitic steel type 304, where the flux was placed and heated but not soldered. The evidence resulting from the addition of fluorine^{18} to the flux indicated that fluorine^{18} remained between the solder and the rustless alloy after the joint was completed. Islands of varying concentrations of fluoride salts were deposited at the interface between the rustless alloys and the solder. It would appear likely that crevice corrosion is initiated through solution of salts in the area of this deposition.

M23. Milestones in the Development of Modern Dental Casting Investments.—*John S. Shell, The Ransom & Randolph Company, Toledo, Ohio.* There have been a number of notable milestones in the development of present-day casting investments and, although the fundamentals of the process have come down from antiquity, the present type of investment is of relatively recent origin. The history of investment casting prior to the work of Dr. Taggart is interesting, but not especially illuminating. The difference between the composition of Taggart's investment and present-day investments is principally a matter of minor, although important, addition agents—with two possible exceptions: (1) the introduction of cristobalite used in place of quartz; and (2) substitution of hydrocal or densite for plaster of Paris. The milestones might be divided into three groups: (a) the developments by manufacturers for which patents have been obtained, such as Weinstein's introduction of boric acid, Moore's introduction of chlorides, Coleman and Weinstein's use of cristobalite, Moore's use of organic material to produce a reducing atmosphere, and Prange's introduction of ethyl silicate; (b) the discoveries by the dental profession of the value of various properties of dental investments, originating with Taggart's work, followed by Scheu with hygroscopic expansion, the control of hygroscopic expansion by Hollenback and Peyton, and the minimizing of hygroscopic expansion as suggested by Phillips; and (c) the present attitudes toward investments as indicated by suggested changes in A.D.A. Specifications by the Committee on Revised Specification No. 2.

M24. The Effect of the Microstructure on the Physical Properties of Chromium-Cobalt Alloys.—*Kamal Asgar, University of Michigan, Ann Arbor.* The effect of different casting conditions on the grain size of the cast chromium-cobalt alloys was studied. Also the physical properties of cast pieces having different microstructures were determined. The relation between the microstructure and the physical properties was established. After being etched, the cast cobalt base alloys were subjected to a bend test. In this way, a comparison was made of the relative ductility of the matrix material and the carbide phase present in the cast structure. Carbide phase had very little ductility and, whenever carbides were continuous, the cast structure had a very low elongation. By varying the casting conditions, the shape as well as the amount of the carbide phase could be changed. In this manner, the physical properties of the alloy within the limits could be controlled.

M25. Method of Determining the Noble Metal Content of Dental Gold Alloys.—*Harold J. Caul, American Dental Association Research Division, National Bureau of Standards, Washington, D. C., and W. Stanley Clabaugh, National Bureau of Standards, Washington, D. C.* The sample is decomposed with diluted aqua regia and the insoluble silver chloride is separated. After adjusting the acidity of the solution to pH 3 with sodium hydroxide, the gold is precipitated as metal with sodium nitrite and, at the same time, the platinum and palladium are converted to soluble complex nitrites. The base metals are precipitated as the hydrated oxides by making the solution slightly alkaline with sodium hydroxide. After removing the base metals, just enough sulfuric acid is added to the filtrate to convert the nitrites and nitrates to sulfates. The resulting solution is evaporated to dryness. The residue is dissolved in distilled water. The solution, which is made slightly alkaline by adding sodium hydroxide, now contains only the platinum metals which can be precipitated collectively with sodium formate. The precipitate of the platinum metals will coagulate completely if the solution is alkaline. The precipitates of the gold and of the platinum metals may be weighed together and constitute the noble metal content of the alloy. The method makes some

sacrifices to accuracy for the sake of speed. Iridium, if present, will be found with the silver chloride. Iridium, usually absent in dental gold alloys, rarely amounts to more than 0.1 per cent. About 1 mg. or less of palladium may be precipitated with the base metals. Both iridium and palladium can be recovered if a higher accuracy is desired.

M26. A STUDY OF THE ACCURACY OF LARGE DENTAL CASTINGS.—*Bjorn Hedegard, E. W. Skinner, and R. Earnshaw, Northwestern University Dental School, Chicago.* The percentage errors were measured in castings simulating dental castings including two saddles and a palatal bar as compared with the dimensions of an Invar master model. It was demonstrated that the main source of the error could be attributed to the hygroscopic expansion of the investment model duplicated by means of a hydrocolloid duplicating material. Measurements of the castings indicated that significant changes in dimension occurred after the sprues were removed. Such changes were attributed to stress release as a result of the method of spruing. Further dimensional changes of less magnitude occurred when the gold castings were annealed. It was estimated that the casting shrinkage of the gold castings was slightly overcompensated during the heating of the investment, but the casting shrinkage of the chrome-cobalt alloy was undercompensated.

M27. PORCELAIN FUSED TO GOLD.—*Denton L. Smith, Richard L. Coleman, and Roy Wain, J. M. Ney Company, Hartford, Conn.* The process of fusing porcelain directly to a gold alloy is one method of combining the esthetics of porcelain and the strength of a gold alloy for use in prosthetics. The methods of determining the properties of the porcelain are discussed, together with the results of the tests. Indications are that the porcelain has adequate strength and that the bond between the porcelain and gold alloy is stronger than the porcelain itself. Satisfactory results from the use of porcelain fused to gold lies in the correct combination of the two materials. The bulk and shape of the casting must complement the properties of the porcelain and lend strength to it. This is accomplished by applying simple but necessary principles to the construction of veneers and crowns. When these principles are followed, satisfactory performance of the material combination is obtained.

M28. EFFECT OF OPAQUE LINERS ON SHADE OF PORCELAIN VENEERS.—*John F. Johnston, Jorge von Mohr, and R. W. Dykema, Indiana University School of Dentistry, Indianapolis.* The influence of opaque liners of varying shades on fused porcelain veneers, compounded of powders designed to form a matured veneer of a predetermined shade, was observed and noted empirically by eye and then measured using the Hunter color and color-difference meter. The end result was predicted in advance, and shades and amounts were used which it was thought would give the desired effect. Accuracy, or the margin of error, was registered or traced with the meter. Changes in the quantities and shades were instituted on the basis of comparative readings. Many shade formulas were worked out and duplicated consistently; a few seemed impossible to attain with the materials available.

M29. COLOR STANDARDS FOR SILICATE CEMENTS.—*George Dickson, and Philip Oglesby, National Bureau of Standards, Washington, D. C.* A procedure for measurement and specification of the colors of silicate cements in terms of tristimulus values was developed. The method is based on the use of a tristimulus color-difference meter in combination with a series of calibrated porcelain standards covering the range of colors of the cements. Six porcelain standards are used to determine a linearity or calibration curve for the color-difference

meter on which measurements of the cement are to be made. Silicate specimens are measured while immersed in water in a cell with a glass bottom through which the specimen is illuminated. Results indicate that with careful control of the factors affecting the stability of the color-difference meter and control of the dimensions and area of illumination of the specimens, the precision of color measurements is equal or greater than the precision with which duplicate specimens can be made. Data on the tristimulus values of most of the widely distributed brands and shades of cement will serve as a basis for recommendations for specifications for the colors of silicate cements.

M30. Utilization of the Water Turbine Principle in Crown Preparation.—*Philip M. Steen, New York, N. Y.* With the advent of high speed into dentistry, many interesting phases of reducing tooth structure are unfolded. One of these is the preparation of jacket and veneer crowns using the water turbine instrument. Due to the fact that the water turbine rotates at speeds from 45,000 to 50,000 r.p.m., a large amount of the noise, pain, and vibration of the conventional drill is lost. Furthermore, the instrument will stop if it gets jammed between two teeth, thereby providing a safety factor. Higher speed is not necessary, as sufficient torque is developed with the water, as compared with air which requires a greater number of revolutions per minute to attain the same amount of torque for cutting purposes. The jacket and veneer crown are prepared using four or five diamond stones, and a precise number of steps. This procedure requires considerably less time to complete. The average time to complete a molar preparation with the conventional instrument is about 30 minutes, and for an anterior tooth (central), 25 minutes. With the water turbine instrument, actual timing shows that the average time to complete a molar preparation is between 14 and 15 minutes and an anterior tooth, between 10 and 12 minutes. A smooth, polished finish is automatically attained. On over 500 full-crown preparations and approximately 400 two- and three-surface gold inlays, fillings, etc., no pulpal involvement was noted within a period of 1½ years. On quite a few preparations, little or no Novocaine was necessary for inlay and filling preparations. Experiments have shown that, with the use of the water turbine instrument, the nervous tension usually associated with high speeds is considerably lessened, and it is being welcomed by the patient as materially decreasing the discomfort caused by heat, noise, and vibration during tooth preparation.

M31. Operating Characteristics of High-Speed Dental Handpieces Under Load.—*R. L. Lasater, E. W. Skinner, and R. S. Lindenmeyer, Northwestern University, Chicago.* Three operating characteristics (noise level, torque, and speed) of a number of high-speed dental handpieces were compared with the similar characteristics of a high-speed cutting head designed for testing the cutting effectiveness of dental burs at constant torque and variable load and speed. The three characteristics studied were found to be interrelated; the torque decreased with increase in speed, and a definite increase in noise level occurred with increased speed. In the dental units, the noise level reached the pathologic limit of 90 decibels in some cases, whereas at the high speeds (146,000 r.p.m.), reached with the specially designed constant torque cutting head, the noise level was 114 decibels. In belt-driven handpieces, the maximum speed attained under load was much less than that claimed, because of low torque and belt slippage at high speeds.

M32. Noise From High-Speed Handpieces.—*Vernon R. Manny, Kenneth R. Cantwell, University of Oregon Dental School, and Archie R. Tunturi, University of Oregon Medical School, Portland.* There has been some concern that

noise from high-speed dental handpieces may attain sufficiently high levels to produce hearing loss among patients or dentists. The noise of concern in this report is the air-borne noise arising from the instrument and reaching the ear by air around the head. The noise level measurements were made on two makes of instruments: Eleven (1 used and 10 new) Ritter R-Borden Airotors and 6 used Page-Chayes instruments. The measurements were made in an irregular-shaped operating room with painted walls and fiberglass acoustical tile on the ceiling. All measurements were made with a general radio type 1551-A sound level meter and a general radio type 1550-A octave band noise analyzer. The microphone was maintained at a distance of 8 inches from the instruments tested. The tests were made with the instruments free-running without bur, with bur, and while cutting tooth structure. The maximum levels encountered with the Page-Chayes instruments were approximately 70 to 75 db. Approximately one half, or less, of the Ritter R-Borden Airotors produced noise levels above the minimum risk level. However, there appeared to be no hazard to the patient or dentist due to air-borne sound, since the risk levels are based on an 8-hour day for a lifetime. There is little chance that the average dentist would be exposed to the noise for an average of over 45 minutes per day.

M33. EFFECT OF COOLANTS ON TEMPERATURE RISE RESULTING FROM CAVITY PREPARATION.—*Arthur W. Aplin, Kenneth R. Cantwell, and Vernon R. Manny, University of Oregon Dental School, Portland.* Clinically and via personal reports, we have not observed the gross deleterious effects, attributed to the use of air as a coolant with operating speeds in excess of 100,000 r.p.m. and light pressure. The specimens for examination were noncarious human teeth extracted for periodontal, prosthetic, or orthodontic reasons. One hundred and fifty clinically acceptable cavity preparations of the Class II and Class V design were cut, using the Leeds and Northrup Azar recorder and a No. 28 gauge chromel-alumel thermocouple to record temperature changes during tooth removal; the Page-Chayes and Borden (Ritter) Airotor handpieces operating at 145,000 and 280,000 r.p.m., respectively; No. 701 carbide burs; 1 to 2 ounces continuous pressure (lateral or vertical); and air (72° to 78° F.) as a coolant. The prepared teeth were sectioned in a manner to provide a profile of the thermocouple and cavity preparation. The maximum range of temperature rise resulting from Class II and Class V preparations was 15° to 50° F. and 18° to 28° F., respectively. Examination of the thickness of dentin, which insulated the thermocouple from the bur and the corresponding maximum temperature rise, revealed the following: (a) no observable relationship for Class II preparations, and (b) between 1.8 to 0.3 mm. separation of thermocouple and bur for Class V preparations. It was felt that further study is required to provide a more concise picture of the above temperature ranges, albeit they may suggest circumstances whereby air might be used as a coolant.

M34. RELATION OF STRUCTURE TO MICROHARDNESS OF HUMAN DENTINE.—*R. G. Craig, P. E. Gehring, and F. A. Peyton, University of Michigan, Ann Arbor.* Microhardness measurements were taken in areas on transverse sections of freshly extracted mature teeth on the basis of the amount of stain taken up on an adjacent section, using a modified Pollak trichrome staining technic. Knoop hardness numbers obtained on transverse crown sections showed that a ring of dentine about 0.5 mm. in width, adjacent to the dentinoenamel junction, was approximately 15 Knoop hardness numbers less than the

surrounding dentine (KHN = 65 – 72). This difference may be attributed to the presence of interglobular dentine. Transparent dentine was observed in several of these sections and was found to be about 10 Knoop hardness numbers harder than the adjacent dentine. Microhardness measurements in transverse root sections showed that the dentine near the pulp chamber was softer than adjacent dentine, having a Knoop hardness number in the order of 35. The soft region near the pulp chamber may be explained on the basis of porosity and/or the presence of predentine. The hardness of the root dentine increased and reached a stable value similar to crown dentine at about 0.5 mm. distant from the pulp chamber. Several sections contained carious areas and it was observed that the area adjacent to the caries had microhardness values approximately 10 Knoop hardness numbers greater than the surrounding dentine.

M35. Compressive Properties of Tooth Tissues and Restorative Materials.—*John W. Stanford, Keith V. Weigel, and George C. Paffenbarger, American Dental Association, Dental Research Section, National Bureau of Standards, Washington, D. C.* A refined method of preparation of cylinders of enamel and dentine over that previously reported was developed. The method entails the use of a jeweler's lathe and a hydraulic handpiece. In addition to the hard tooth tissues, cylindrical specimens of silicate cements, zinc phosphate cements, amalgam alloys, and direct filling resins approximating the size of the samples of tooth structure, were tested. The results show that the compressive properties of the restorative materials ranged from 0.4×10^6 to 3.3×10^6 psi for modulus of elasticity, from 6,400 to 30,000 psi for proportional limit, and from 11,000 to 57,800 psi for compressive strength. Values for human dentine varied from 1.4×10^6 to 2.9×10^6 psi for modulus of elasticity, from 16,000 to 22,900 psi for proportional limit, and from 31,700 to 50,000 psi for compressive strength, depending upon the type of tooth and original location of specimens in the tooth. Values for human enamel varied from 2.8×10^6 to 8.8×10^6 psi for modulus of elasticity, from 11,800 to 46,500 psi for proportional limit, and from 16,500 to 54,600 psi for compressive strength, depending upon the type of tooth and original location of the specimen in the tooth.

M36. An Investment Compounded Specifically for Ceramic Procedures.—*K. N. Morrison, and M. E. Warnick, University of Washington, Seattle.* This paper describes an effort to simplify the construction of ceramic restorations by the elimination of the need for a platinum matrix. This was accomplished by baking porcelain directly into a mold of "high heat" investment. The mold itself was formed by vibrating the fluid investment into a rubber base or compound impression of the prepared tooth. These procedures were simplified to the point where porcelain inlays could be fabricated with no more difficulty than is required to make a simple gold inlay. A quartz investment with an ethyl silicate binder proved most readily adaptable for ceramics for the following reasons: (1) green strength of the setting investment was adequate, (2) this investment reproduced fine cavity detail, (3) it would withstand normal manipulation of poreclain without loss of detail, and actually made condensation easier, (4) thermal expansion is adjustable and could be made to compensate for porcelain shrinkage and the small setting contraction of the investment itself, and (5) this material could be readily removed from the porcelain after firing. We are presently using the investment at the University of Washington and it is producing very gratifying results.

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INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

Proceedings of the Thirty-Seventh General Meeting
Sheraton-Palace, San Francisco, California
March 19, 20, 21 and 22, 1959

EXECUTIVE PROCEEDINGS

(Compiled by Dan Y. Burrill, Secretary-Treasurer)

The Thirty-Seventh General Meeting of the I.A.D.R. opened with a meeting of the Council at 9:00 A.M., Thursday, March 19, 1959. On each following day of the meeting, the Council met from 12:20 P.M. until just before the afternoon scientific session.

COUNCIL ACTIONS

The use of I.A.D.R. membership lists for commercial purposes was discussed at length. The Council voted that the list should not be sent to any commercial user for any purpose. This applies to railroads and airlines as well as others. No commercial or advertising use will be allowed.

There were some objections to the omission of the Read-by-Title section of the program at the 1959 meeting. After discussion it was voted that there be no Read-by-Title section in the 1960 program. Authors who would send abstracts for this section are urged to submit short notes or papers to the editor for early publication in the short notes section of the Journal of Dental Research.

The Membership Committee approved 80 persons for election to membership in the Association. The Council accepted all nominations.

The Committee on Program Arrangements reported the following recommendations for the 1960 meeting:

1. Not more than 250 papers should be accepted for the general program (not including papers for the Dental Materials Group program or for symposia).

2. Two or three symposia should be arranged, in addition to the usual scientific sessions.

3. November 30, 1959, will be the deadline for receipt of abstracts for the 1960 meeting.

4. Abstracts not conforming to specifications will not be accepted, but will be returned to the authors.

5. Abstracts of papers presented at the meeting will be published in the Journal of Dental Research after the meeting.

6. Preprinted abstracts to consist of material accepted for the program, as presented by the author(s) without corrections, are to be sent to every member of the Association before the meeting.

   a. These preprinted abstracts should be marked "preliminary—not for quotation or citation."

   b. Authors' names, but not titles, degrees, or locations will be given.

Association dues for 1959-1960 were set as follows:

North American Division members—$15.00 ($7.00 for the subscription to JDR and $8.00 for the general fund).

Members outside North America—$3.00 dues. Subscription to JDR, if desired, will be $7.00 plus postage additional.

The Council approved the Editor's report (Supplement B), the membership of the Editorial Board suggested by the Editor, and the measures proposed by him relating to the publication of the JOURNAL.

The Committee on the E. H. Hatton Award recommended revisions of the rules of the contest. The report was approved and adopted as Council action and appears in Supplement C.

Dr. Genevieve Roth and Col. D. C. Hudson were elected to the ad interim committee of the Council.

At the request of the Secretary-Treasurer, a committee was appointed (L. S. Fosdick, H. T. Knighton, H. B. G. Robinson, and D. Y. Burrill) to investigate the possibility of employing an executive secretary to carry on the routine business of the secretary's office.

EXECUTIVE SESSION I. March 19, 1959. 8:00 P.M.

The minutes of the 1958 Meeting, appearing as the report of the proceedings in the JOURNAL OF DENTAL RESEARCH, Vol. 37, No. 5, 1959, were approved as printed.

Doctors Costich, Mandel, and Porter were appointed tellers to count the votes for officers and on the proposed amendments to the Constitution and By-Laws.

Greetings were read from Dr. E. H. Hatton and from Deans Fleming and Tocchini. Messages from the Association were sent to Dr. Hatton and Dr. Harold Hillenbrand.

The Secretary-Treasurer presented an audited report of the finances of the Association. The report was received and approved and is reproduced in the Supplement (D and E).

President Williams reported for the ad interim committee of the Council as follows:

A grant was received from the National Institute of Dental Research to assist three selected men in attending the International Congress of Physiology and Pharmacology in Buenos Aires, August, 1959. Nominations were sought and the following were selected to attend: Bernard K. Forscher, University of Kansas City; Leon Kraintz, University of Texas; Frederick M. Liebman, New York University.

A grant of $30,000 was received from The Colgate-Palmolive Company for the support of a conference in conjunction with the meeting of the American Dental Association in New York, September 7, 8, 9, 1959. The grant funds are to be used primarily to assist participants from other countries in attendance at the conference.

As a representative of the Association, President Williams was invited to attend a meeting with Secretary Flemming and Surgeon General Burney of the Department of Health, Education and Welfare on December 10, 1958. The purpose of the meeting was to explore current and long-term needs in research and how results of research may be most effectively used in the interest of health information and education. The specific topics discussed were: Training of personnel and support for same; recruiting personnel and exchange of personnel between research institutions; fellowships for student research during summers; and availability of research findings, i.e., exchange of information, how to find information, pooling information, and screening efforts.

The Association voted to express its support of the proposal by Senator Hubert H. Humphrey for an International Public Health and Medical Research Year, and its support of Senator Lister Hill's proposal to establish a National Institute of International Research at the National Institutes of Health (Supplement H).

EXECUTIVE SESSION II. March 22, 1959. 4:00 P.M.

The meeting convened as a meeting of the North American Division of the I.A.D.R. Dr. Seymour Kreshover was elected representative on the Council of the A.A.A.S. and Dr. Albert Dahlberg was elected representative to Section Nd of the A.A.A.S. The meeting adjourned and immediately reconvened as a general meeting of the I.A.D.R.

The schedule of meeting places previously recommended by the Committee on Meeting Places, and approved by the Council, was reported to the Association as follows:

1960—Chicago, Ill.—Morrison Hotel
1961—Boston, Mass.—Statler Hotel
1962—St. Louis, Mo.—Jefferson Hotel
1963—Pittsburgh, Pa.—Penn-Sheraton Hotel
1964—Undecided
1965—Toronto, Canada—Royal York Hotel

The tellers reported that the officers nominated by the Council were elected, that the amendment to the Constitution was adopted by a vote of 262 to 122, and the amendment to the By-Laws by 274 to 114 (Supplement G).

An amendment to the By-Laws providing for honorary memberships was presented by a committee previously appointed for the purpose (B. G. Bibby, W. Lefkowitz, and B. Z. Rabinowitch) and was adopted unanimously (Supplement G).

An amendment to the By-Laws authorizing the Editor to co-sign checks drawn on the funds of the JOURNAL OF DENTAL RESEARCH was proposed and adopted by unanimous vote (Supplement G).

An amendment to the By-Laws, Section A, interpreting the newly adopted amendment to the Constitution and By-Laws concerning membership, was proposed by Dr. H. A. Zander on behalf of a group of members. After extended discussion the amendment was adopted, with four dissenting votes (Supplement G).

Resolutions unanimously adopted by the Association appear in the Supplement H.

The Evanston State Bank and Trust Company of Evanston, Illinois, was approved as repository for the funds of the Association for the coming year.

The Association unanimously approved the payment by the Secretary-Treasurer of all expenses of the 1959 Meeting.

The following members were unanimously elected Directors of the I.A.D.R. (a corporation not for profit incorporated under the laws of the State of Illinois): Paul E. Boyle, Reidar F. Sognnaes, Ned B. Williams, H. B. G. Robinson, Holmes T. Knighton, James A. English, Frank J. Orland, Thomas J. Hill, and Dan Y. Burrill.

A budget for the office of the Secretary-Treasurer for 1959-1960 was submitted and unanimously approved. Unanimous approval was also given for the establishment of a contingency fund for the use of the Editor of the JOURNAL OF DENTAL RESEARCH and for a contribution toward the cost of publishing in the JDR the abstracts of papers presented at the 1959 Meeting (Supplement F).

The names of 80 persons nominated by the Council for election to membership in the Association were read. By unanimous vote, the Secretary was instructed to cast a single ballot for their election (Supplement I).

The officers newly elected for the coming year were installed and President Robinson took the chair. Past-president Williams received a vote of thanks for his accomplishment during his year as president.

The meeting adjourned sine die.

During the 1959 Meeting, 255 scientific papers were presented. In addition, there were two symposia, one on "Electron Microscopy in Dentistry" and the other on "Bone Implants" with eight papers in each.

The I.A.D.R. and the American Association of Dental Schools held a joint session on Sunday evening, March 22, 1959. The theme for this meeting was "Impact of Dental Research on the Teaching and Practice of Dentistry" and the program included presentations by the following: Ralph W. Phillips, *Indiana University School of Dentistry;* John B. Macdonald, *Forsyth Dental Infirmary for Children;* Raymond J. Nagle, *New York University College of Dentistry;* Beryl Ritchey, *Colorado Springs, Colo.* A general discussion followed the program.

The annual banquet on Saturday, March 21, 1959, was attended by 144 persons. The incoming president, Dr. Hamilton B. G. Robinson, presented his inaugural address "The Journal of Dental Research 1919-1958" (Supplement to Proceedings, see below). Ralph W. Phillips received the Wilmer Souder Award, the fifth annual presentation of this honor recognizing outstanding contributions in the field of Dental Materials research.

Richard L. Hoffman of the University of Illinois School of Dentistry received the Edward H. Hatton Award for his paper on "Formation of Periodontal Tissues Around Subcutaneously Transplanted Hamster Molars." Honorable mention was given Benjamin F. Hammond of the University of Pennsylvania School of Dentistry for his paper on "Submaxillary Saliva as a Culture Medium."

SUPPLEMENT TO PROCEEDINGS

A. ATTENDANCE AT COUNCIL MEETINGS. The following were present at one or more of the four meetings. S. S. Arnim, G. Bevelander, D. Y. Burrill, P. E. Boyle, Mary C. Crowley, E. C. Dobbs, J. A. English, L. S. Fosdick, A. K. Fisher, J. Gibilisco, P. Goldhaber, L. I. Grossman, R. L. Ireland, E. Johansen, E. B. Jump, V. Kalnins, H. T. Knighton, W. Lefkowitz, R. S. Manly, J. C. Muhler, P. L. Munson, F. J. Orland, G. C. Paffenbarger, G. J. Parfitt, B. Z. Rabinowitch, J. R. Ring, H. B. G. Robinson, Genevieve D. Roth, G. Ryge, L. M. Sreebny, N. B. Williams.

B. REPORT OF THE EDITOR. The JOURNAL OF DENTAL RESEARCH is now exactly 40 years old this month of March. As most of you heard in Dr. Robinson's commendable Presidential Address last evening, the JOURNAL has had a long and difficult road in its evolution to become the Association's official publication. There have been changes all along the way, which make the changes in the JOURNAL during this past year seem minor, indeed.

During this past year, there has been a new Editor in action, an extensive manuscript review system has been put into effect and all funds for the operation of the JOURNAL have been placed in the Association's Treasury but in a separate JOURNAL account.

Data on manuscripts are as follows: There were 259 separate items received for publication since I have been Editor. Of this number, 192 were manuscripts (or things resembling manuscripts). The remaining number comprised proceedings of local, divisional, or annual meetings, research notes, and other minor, single page items. Among recently received manuscripts and other articles, it was felt necessary to have about 70 per cent revised to a minor or major degree by the authors. My own personal concept has been to make poorly written manuscripts publishable by author's revision if possible, particularly in the case of the younger contributors and those from abroad. There will have been published a total of 167 scientific manuscripts with the March—April issue now in press. Twenty-five papers were rejected as unsuitable for publication in the JOURNAL. There were 1,186 (arabic numbered) pages in Volume 37 for 1958 as compared to 1,014 for 1957, and as contrasted to 805 for the 1948 Volume 10 years ago, or 532 pages in Volume 17, 20 years ago. There is no question about the fact that we have grown! From the time a paper is first received, until it finally appears in the JOURNAL on your desk, now takes 6 to 7 months and there is not much of a backlog. (Perhaps, upon my arrival home I'll find a great pile of mail containing more manuscripts.)

In order to learn the thinking of the readers of the JOURNAL, a questionnaire was sent to the I.A.D.R. membership last spring. More than half were returned early, but one

came in as late as last week. The results of this questionnaire, while too long to present here, did give the new Editor guidance as to the views of I.A.D.R. members about their JOURNAL and some were quite revealing, I might add.

Since many of you have been authors or potential authors of papers, you may be interested in knowing how a manuscript is processed from the moment it arrives at our editorial office until it appears in your copy of the JOURNAL. Since time and space do not permit this now, later mention will be made of the topic.

A year ago, my predecessor, Hamilton B. G. Robinson, admonished me that the Editor must be somewhat of an autocrat. I was skeptical then; I am less so now! I believe I *am* growing a thick skin. There have been about 1,140 individual pieces of mail received regarding the JOURNAL during the past 12-month period. To try to answer properly even the more important letters has been a terrific effort.

With the one goal in mind of constantly seeking to improve the JOURNAL, there are other changes contemplated for the future. For example, the Publication Committee voted unanimously 2 days ago to give serious consideration to having the University of Chicago Press publish the JOURNAL beginning in 1960. The Editor believes there are strong reasons for such a step which he will be glad to discuss with anyone interested.

Thus, on behalf of the JOURNAL, I wish to thank the many individuals who have helped with the JOURNAL OF DENTAL RESEARCH the past year. This includes the small and part-time staff at the Zoller Clinic, the two Associate Editors (Dr. Macdonald and Dr. Mitchell) and other members of the Publication Committee including the former Editor, Ham Robinson, for occasional suggestions, as well as the several individuals who helped with the JOURNAL at The C. V. Mosby Company in St. Louis. Special thanks go to the manuscript referees, the Editorial Advisory Board, and particularly to that half of them who have reached the end of their one-year term on this Board. It goes almost without saying that all these Association members including the Editor have donated their time and effort to the cause of the JOURNAL.

The JOURNAL OF DENTAL RESEARCH must rely on gifts from outside the I.A.D.R. As almost everyone knows, I trust, your membership subscriptions pay but a small fraction (1/10) of the total cost of the JOURNAL. During this past year, there have been received by the I.A.D.R. Treasury several gifts and grants. On behalf of the Publication Committee I would like to thank The Colgate-Palmolive Company for its annual gift of approximately $17,000 to provide dental student subscriptions; the William J. Gies Foundation for the grant of $3,900 for extra pages; the N.I.D.R., U.S. Public Health Service for a grant of $5,000 for JOURNAL expansion, for use in 1959. We thank the following Supporting Associates for their contributions of $100 or more from each, received in 1959 and only within the last 2 weeks when requests were sent out; more replies are expected in the mail:

Julius Aderer, Inc., New York, N. Y.
Engelhard Industries, Inc., Newark, N. J.
L. D. Caulk Company, Milford, Del.
Crescent Dental Manufacturing Co., Chicago, Ill.
Dentists' Supply Company of New York, York, Pa.
Hanau Engineering Company, Inc., Buffalo, N. Y.
H. D. Justi and Sons, Inc., Philadelphia, Pa.
The J. M. Ney Company, Hartford, Conn.
Precious Metals Research Works, Inc., New York, N. Y.
The Acralite Company, Inc., New York, N. Y.
Ritter Company, Inc., Rochester, N. Y.
Squibb Institute for Medical Research, New Brunswick, N. J.
Kerr Manufacturing Company, Detroit, Mich.

Despite some of these tenebrific facts and figures and efforts, a great many of us firmly believe we have here in our JOURNAL a permanent record for posterity of meritorious and even scholarly achievements in oral research, a principle upon which this Association, I trust, staunchly stands. I thank you!

FRANK J. ORLAND, Editor

C. REVISION OF THE RULES FOR THE EDWARD H. HATTON AWARD COMPETITION. Recommended by a special committee (E. B. Jump, H. T. Knighton, W. T. Sweeney, Chairman) appointed in 1958 to study the rules. Recommendations approved and adopted by the Council.

Preamble: The Hatton Award's purpose is to encourage young investigators to present results of their dental research before the I.A.D.R. so that they may receive the recognition they deserve. The Award is intended to serve as a means of bringing to the attention of young men and women the fact that dental research offers a most challenging field for their talents.

The following rules shall be used in the selection of the Hatton Award winner.

1. The Hatton Award is for novice research workers. A novice for the purpose of this award shall be a person who has not presented a research paper before any national scientific organization, or published a report as principal author in a national scientific journal.

2. The paper must be presented by the novice at the annual meeting of the I.A.D.R.

3. The paper to be considered for the Hatton Award must be the work of one or more novices.

4. Six copies of the paper and six copies of a biographical sketch of each author must be submitted to the Chairman of the Hatton Award Committee through the Secretary of the I.A.D.R. 30 days before the Annual Meeting of the I.A.D.R. for review.

5. The decisions of the Committee in the selection of winners shall be final.

6. Authors who are eligible to compete for the Award should state that fact in a note attached to the abstract submitted to he program committee.

7. The following items will be given consideration by the Committee in judging the paper presented: (a) originality, (b) scientific value, (c) accuracy of data, (d) conclusions drawn, (e) method of presentation, and (f) general competence in the subject.

D. FINANCIAL STATEMENT FOR YEAR ENDED DECEMBER 31, 1958

Office of the Secretary-Treasurer—Chicago, Illinois.*

Fund Balance, January 1, 1958		
On deposit at State Bank and Trust Company, Evanston, Ill.	$5,865.52	
Balance in Savings Account†	243.13	
Less, Due to Petty Cash	(65.86)	
Total Fund Balance		$6,042.79
Receipts		
Subscriptions	$5,425.00	
Dues	5,782.74	
Registration Fees and Sale of Banquet Tickets	3,366.00	
Interest on U.S. Government Bonds	41.40	
Interest earned on Savings Account	4.88	
Grant for Travel Expense to Int. Cong. of Physiol. & Pharma.	2,350.00	
Total Receipts		$16,970.02
		$23,012.81
Disbursements		
Printing and distributing Journal (The C. V. Mosby Co.)	$5,352.67	
Mailing Operations, Postage, and Printing	541.45	
Gratuity to Secretary-Treasurer	500.00	

*Audit by Dale H. Taylor, C.P.A. (Not included above are expenditures for $147.92 which have accrued at December 31, 1958, but which will be included in 1959 disbursements.)

†These Fund Assets were omitted from prior years' statements.

Annual Meeting Expense			
Meeting facilities, banquets, and luncheons	$1,527.39		
Printing programs, badges, etc.	607.08		
Novice Award	100.00		
Projection and Public Address	990.50		
Other Expense and Refunds	77.13	3,302.10	
American Institute of Biological Science dues		100.00	
Transfer to Journal of Dental Research Fund*		1,000.00	
Office Expenses			
President's office	$140.89		
Addressograph file	62.37		
Clerical and professional service	196.75		
Miscellaneous expense	59.22	459.23	
New equipment†		393.42	
Total disbursements			$11,648.87
Fund Balance, December 31, 1958			
On deposit at State Bank and Trust Company			
Checking account		$11,089.08	
Savings account		248.01	
Petty Cash and Receipts not deposited		26.85	
Total Fund Balance‡			$11,363.94

E. REPORT OF JOURNAL OF DENTAL RESEARCH FUNDS§

March 15, 1958 to March 15, 1959

Balance in Fund, March 15, 1958			$ 973.47
Receipts			
Sale of back issues	$ 94.50		
Supporting Associates' contributions	1,800.00		
Gies Foundation Grant	3,900.00		
National Institute Dental Research Grant	5,000.00		
I.A.D.R.—transfer from general fund	1,000.00		
Colgate-Palmolive Co. for 1958 student subscr.	17,036.00		
Colgate-Palmolive Co. for 1959 student subscr.	16,000.00		
Funds forwarded from Indiana Nat'l. Bk. of Indianapolis	191.57		
Total Receipts			$45,022.07
			$45,995.54
Disbursements			
The C. V. Mosby Co. for extra pages	$ 6,418.14		
The C. V. Mosby Co. for student subscriptions	17,031.00		
Miscellaneous expense	86.99		
Total Disbursements			$23,536.13
Balance in Fund, March 15, 1959			
On deposit at State Bank and Trust Company, Evanston, Illinois			$22,459.41

*Represents contribution to the cost of publishing abstracts in JDR.
†Electric typewriter and stand—allowance of $450 in 1958-1959 budget.
‡In addition to the Cash Assets in the Fund, the Association owns equipment which cost $577.19 and U.S. Government Bonds which have a total maturity value of $1,650.
§Audit by Dale H. Taylor, C.P.A.

F. BUDGET FOR 1959-1960

Office of Secretary-Treasurer

Direct Expenses:

Clerical	$ 250	(Note: The amounts in each of the categories of direct expense are approximate. Transfers from one category to another as necessary are approved.)
Postage	150	
Supplies	150	
Printing	600	
Travel and misc.	50	
E. H. Hatton Award	100	
Contingency Fund	200	
Gratuity	500	
Secretarial services	1,500	
Total	$3,500.00	

Contingency fund for Editor of JDR—$1,000.00

Contribution toward cost of publishing abstracts in JDR—$1,000.00

G. AMENDMENTS TO THE CONSTITUTION AND BY-LAWS ADOPTED AT THE 1959 MEETING.

Article III, Section 1 (of the Constitution).—Any individual who is interested in Dental Science and Dental Research shall be eligible to membership in this Association, provided such person conforms to the recognized standards of professional ethics.

By-Laws, Section A-1.—Nominations for membership shall be signed by at least two members of the Association who shall state their reasons for believing the nominee eligible for membership.

By-Laws, Section A-2.—The words "individual who is interested in Dental Science and Dental Research" in Art. III, Section 1 of the Constitution shall be interpreted as meaning (a) persons who are conducting or have conducted research, (b) persons who have presented scientific papers based upon original research before national or international meetings, (c) persons who have had one or more scientific papers based upon original research published in scientific journals.

By-Laws, Section A-7.—Honorary Membership. (A) One honorary member may be nominated each year by unanimous recommendation of the three most recent living past presidents of the Association. Such honorary members shall have all the rights and privileges of membership but shall not receive the JOURNAL. (B) Honorary members shall be selected on the basis of the candidate's significant contributions to the advancement of dental research.

By-Laws, Section G.—Authorized Banks and Expenditures. 1. Funds of the Association shall be deposited in a bank or banks approved for the purpose by the Association. Authorized expenditures from the general funds of the Association shall be made by checks each of which must be signed by the President or President-elect and the Secretary-Treasurer or Assistant Secretary-Treasurer. 2. Expenditures from the funds of the JOURNAL OF DENTAL RESEARCH of the Association shall be made by checks each of which must be signed by the Editor, the President, or the President-elect, and by the Secretary-Treasurer or Assistant Secretary-Treasurer.

H. RESOLUTIONS UNANIMOUSLY ADOPTED.

1. Resolved: That the I.A.D.R. express its appreciation to Dr. and Mrs. Russell D. Coleman, the members of his local arrangements committee, their wives, and the Dental

Hygienists of the University of California School of Dentistry, all of whom aided in making the 1959 meeting a successful one.

2. The International Association for Dental Research, assembled at its Thirty-Seventh General Meeting, March 19-22, 1959, in San Francisco, unanimously endorses the Senate Joint Resolution 38 for the establishment of a National Institute for International Health Research and offers its assistance in implementing the passage of this "Health for Peace" proposal.

3. Be it resolved that the International Association for Dental Research support Senator H. H. Humphrey in his efforts toward establishment of an International Public Health and Medical Research Year.

I. ELECTIONS TO MEMBERSHIP, March 21, 1959.

NORTH AMERICA.—Leonard A. Altemus, *Washington, D. C.;* D. H. Anthony, *Ann Arbor, Mich.;* Herbert D. Ayers, Jr., *Flushing, N. Y.;* Paul N. Baer, *Bethesda, Md.;* William H. Bell, *Houston, Tex.;* Michael Buonocore, *Rochester, N. Y.;* Thomas C. Cheng, *Baltimore, Md.;* Sidney A. Cohn, *Memphis, Tenn.;* Robert G. Craig, *Ann Arbor, Mich.;* Josse E. deWever, *Jersey City, N. J.;* Frank J. Draus, *Pittsburgh, Pa.;* William H. Emerson, *Boston, Mass.;* H. Cline Fixott, Jr., *Portland, Ore.;* Viola D. Flanagan, *Kansas City, Mo.;* Shirl S. Fox, *Palo Alto, Calif.;* H. William Gilmore, *Indianapolis, Ind.;* Roland R. Hawes, *Rochester, N. Y.;* Alvin C. Hileman, *San Francisco, Calif.;* A. M. Hunt, *Toronto, Canada;* P. M. Jones, *Kansas City, Mo.;* J. Stanley Jordan, *Philadelphia, Pa.;* Krishan K. Kapur, *Boston, Mass.;* Arthur A. Kiryati, *Rochester, N. Y.;* I. Kleinberg, *Winnipeg, Canada;* Donald E. Knapp, *Ann Arbor, Mich.;* Allen J. Koslin, *Birmingham, Ala.;* F. Earle Lyman, *Bethesda, Md.;* Thomas A. McFall, *Washington, D. C.;* Richard S. Mackenzie, *Ann Arbor, Mich.;* Genowefa T. Mizera, *Chicago, Ill.;* Everett D. Mumaw, *APO New York;* Benjamin Patur, *Hartford, Conn.;* Harry H. Postle, *Columbus, Ohio;* D. Vincent Provenza, *Baltimore, Md.;* Norton M. Ross, *Baltimore, Md.;* Russell Ross, *Seattle, Wash.;* Nathaniel H. Rowe, *St. Louis, Mo.;* Robert G. Schallhorn, *Ft. Ord, Calif.;* Philip Schuback, *Providence, R. I.;* John O. Semmelman, *York, Pa.;* Marvin M. Stark, *Santa Clara, Calif.;* Ronald Steg, *Jamestown, N. Y.;* William F. Talbot, *Detroit, Mich.;* Clifford L. Whitman, *Hackensack, N. J.;* John R. Wilson, *Columbus, Ohio;* Richard A. Winer, *Salem, Mass.;* Joel Wisotzky, *E. Brunswick, N. J.;* Peter Kai-Jen Yen, *Boston, Mass.*

GREAT BRITAIN.—John Neil Anderson, Donald Thomas Bennett, Geoffrey Charles Blake, Peter Burke, Bertram Cohen, John Eric Eastoe, John Hamilton Farrell, P. J. Holloway, Samuel A. Leach, A. G. Leaver, Dorothy A. Lunt, John Walford McLean, Gordon Robert Seward.

JAPAN.—Masatoyo Akiyoshi, Shimpei Araya, Goro Ishikawa, Yasuo Kanzawa, Yojiro Kawamura, Masanao Kidokoro, Yoshio Matsushita, Isamu Nakazawa, Toshihiko Nikuni, Keiichi Tonogai, Tadashi Ueno, Yoshio Watanabe, Hiroshi Yamashita.

OTHER COUNTRIES.—Andrea Benagiano, *Italy;* Ulisse Bifano, *Italy;* Romulo Luis Cabrini, *Argentina;* Björn Hedegard, *Sweden;* John A. M. Jansen, *Malaya;* Stig L. G:son Ostlund, *Sweden;* Maria Schug-Kösters, *Germany;* Mohamed Zahran, *Egypt.*

J. MISCELLANEOUS

1. *Statistics*

Current membership—1,003

North America—804
Outside N.A.—199

Attendance at 1959 Meeting:

Members	293
Nonmembers	280
Guests	118
Total	691

2. *Necrology*

Harold K. Box
Albert J. Broughton
Percy L. Johnson
Arthur Kirschbaum
Harry H. Shapiro
William G. Skillen
Jacob M. Wisan

3. *Officers elected for 1959-1960*

President	Hamilton B. G. Robinson
President-elect	Holmes T. Knighton
Vice-president	James A. English
Secretary-Treasurer	Dan Y. Burrill
Publication Committee (two-year term)	John B. Macdonald
Councillors-at-large	Genevieve Roth
	Lucien A. Bavetta

FURTHER OBSERVATIONS ON THE CARIOSTATIC EFFECT OF PHOSPHATES

F. J. MC CLURE AND A. MULLER, JR.

National Institute of Dental Research, National Institutes of Health, Bethesda, Md.

TWO previous publications have presented our results on the effect of Na_2HPO_4 and $CaHPO_4$ on dental caries in white rats.[1, 2] In this report, there are additional data pertinent to the cariostatic action of $CaHPO_4$ and new data regarding precipitated basic calcium phosphate* and $Ca(H_2PO_4)_2 \cdot H_2O$. There are results also on the effect of intubated phosphate.

EXPERIMENTAL

The regimen of these experiments and the caries diagnosis follow the planning of our previous studies on wheat cereal diets and phosphate supplements.[1, 2] The data are presented in Tables I and II. The basal diets for Experiments 297-306, 310, 311, 273, and 274 (Table I) contained whole wheat flour 80.0 per cent, cerelose 18.0 per cent, and liver powder 2.0 per cent. Minerals for the test diets replaced an equal quantity of wheat flour. In Experiments 297 to 301, sufficient $CaCO_3$ was added to equalize the calcium content of all diets at approximately 0.5 per cent (see Table I). Other mineral supplements are indicated in Table II. For Experiments 318 to 322, wheat flour and lactose in a 10 to 1 ratio were thoroughly mixed, placed in an open pan about 2 inches deep, and autoclaved at 15 pounds pressure for 15 minutes. This autoclaved mixture was then dried and ground to a flour. Two per cent of liver powder was added to complete the diet. The mineral supplements, namely 1.75 per cent $Ca(H_2PO_4)_2 \cdot H_2O$, 2.25 per cent NaCl, and 1.37 per cent $NaHCO_3$ in Experiments 318 to 322 replaced an equal quantity of the whole wheat flour-lactose mix. Selection of these minerals for this particular study was based on their presence in self-rising flours—although a commercial preparation of anhydrous monobasic phosphate is used most extensively for this purpose.†

The diet for Experiments 275 and 276 (Table II) contained 80 per cent shredded wheat biscuit ground to a flour, 18 per cent cerelose, and 2 per cent liver powder. In addition, all the rats received, by medicine dropper orally, once a week a vitamin supplement providing approximately 1,500 units of A, 105 units of D, and 5 mg. of E weekly. Ash, calcium, phosphorus, and protein contents of the diets are shown in Table I.

Received for publication Jan. 12, 1959; revised by authors Feb. 23, 1959.

*Analytical reagent grade, labeled $Ca_3(PO_4)_2$.

†The extent of this use is indicated by the following information supplied by a commercial manufacturer: anhydrous $Ca(H_2PO_4)_2$ in self-rising flour, 1.60%; phosphated flour, 0.35%; pancake and waffle mixes, 1.85%; biscuit mixes, 0.35%; and self-rising cornmeal, 1.64%.

TABLE I
ASH, CALCIUM, PHOSPHORUS, AND PROTEIN ANALYSIS OF DIETS (PER CENT)

	EXPERIMENT NUMBER															
	297 301	298	299	300	302 304	303	305 306	310 311	273	274	275	276	318	319	320	321 322
Ash	2.37	3.65	2.87	2.57	1.39	3.78	3.39	1.47	2.99	1.28	2.90	1.36	1.74	2.96	4.42	5.11
Ca	0.52	0.53	0.54	0.49	0.04	0.04	0.58	0.05	0.52	0.05	0.50	0.03	0.04	0.23	0.27	0.28
P	0.32	0.63	0.64	0.63	0.32	0.85	0.71	0.33	0.38	0.29	0.41	0.32	0.40	0.72	0.78	0.78
Protein*	13.7	13.6	13.7	13.7	14.4	13.7	13.7	14.4	13.1	14.4	10.0	10.0	16.3	15.6	15.0	15.0

*N × 6.25.

The basal diets unsupplemented are extremely deficient in calcium and low in phosphorus, that is, as little as 0.03 per cent calcium and 0.29 per cent phosphorus. Protein is in about optimum quantity for growth except in Diets 275 and 276 in which there is only 10 per cent protein. The lysine inadequacy of wheat protein is well known and becomes a growth-limiting factor.[3] As shown by the data for average daily gain (Table II), the diets are not adequate for normal growth. With a calcium supplement, maximum growth rarely exceeded 1.8 grams daily and with unsupplemented diets was as low as

TABLE II
RESULTS OF STUDIES ON EFFECT OF VARIOUS PHOSPHATES ON SMOOTH SURFACE CARIES DEVELOPED IN WHITE RATS BY WHEAT CEREAL DIETS

EXPER. NO.	MINERAL SUPPLEMENTS IN THE DIET OR INTUBATED	NO. RATS	AVG. DAILY GAIN (GM.)	RATS WITH CARIES (%)	CARIOUS TEETH (NUMBER PER RAT)	CARIES SEVERITY (SCORE PER RAT)
297	Control – $CaCO_3$* (diet)	37	1.0	81.0	2.97 ± 0.37	5.57 ± 0.84
298	Na_2HPO_4 – 1.5% (diet)	39	1.3	5.1	0.05 ± 0.04	0.08 ± 0.06
299	$CaHPO_4$ – 1.5% (diet)	38	0.8	92.1	3.76 ± 0.34	8.42 ± 0.06
300	$Ca_3(PO_4)_2$† – 1.5% (diet)	37	0.8	97.3	4.67 ± 0.59	10.51 ± 0.85
301	Na_2HPO_4 (intubated)	40	1.3	55.0	1.30 ± 0.24	2.40 ± 0.58
302	Control	36	0.9	88.9	3.22 ± 0.33	6.39 ± 0.91
303	Na_2HPO_4 – 2.0% (diet)	30	0.7	16.7	0.33 ± 0.16	0.47 ± 0.22
304	NaCl – 2.0% (diet)	28	1.1	85.7	2.71 ± 0.33	5.21 ± 0.81
305	$CaHPO_4$ – 2.0% (diet)	35	1.0	91.4	3.34 ± 0.31	6.06 ± 0.81
306	$CaHPO_4$ – 2.0% – NaCl 2.0% (diet)	40	1.4	42.5	1.13 ± 0.25	1.75 ± 0.44
310	H_2O (intubated)	37	0.7	100.0	4.57 ± 0.26	10.59 ± 0.99
311	Na_2HPO_4 (intubated)	37	1.0	91.6	3.05 ± 0.29	6.13 ± 0.76
273	Na_2HPO_4 0.40% – $CaCO_3$ 1.25% (diet)	40	1.6	47.5	0.98 ± 0.22	1.80 ± 0.47
274	$Na_2HPO_4 \cdot CaCl_2$ (intubated)	40	1.4	92.5	3.85 ± 0.29	7.25 ± 0.70
275	Na_2HPO_4 0.41% – $CaCO_3$ 1.25%	39	1.3	64.1	1.38 ± 0.22	2.69 ± 0.46
276	Na_2HPO_4 – $CaCl_2$ (intubated)	39	1.2	97.4	4.28 ± 0.26	13.69 ± 1.61
318	Control	37	0.6	100.0	3.72 ± 0.29	8.49 ± 1.10
319	$Ca(H_2PO_4)_2 \cdot H_2O$, 1.75% (diet)	33	0.7	78.7	1.64 ± 0.22	2.85 ± 0.44
320	(Diet 319) + 2.25% NaCl (diet)	39	0.9	28.2	0.38 ± 0.11	0.41 ± 0.12
321	(Diet 320) + 1.37% $NaHCO_3$ (diet)	40	1.4	27.5	0.43 ± 0.12	0.48 ± 0.15
322	Same as Diet 321‡	39	1.0	10.2	0.13 ± 0.06	0.18 ± 0.10

*$CaCO_3$ was added to all diets in this series of experiments. See Table I for analytical data.
†Precipitated basic calcium phosphate.
‡Minerals were present during autoclaving in Diet 321, but not in Diet 322

0.5 to 0.6 grams daily (Diet 318). A microbiological assay of these basal control diets gave the following lysine results expressed as milligram per gram of diet: Diet 297, 3.52 mg.; Diet 302, 3.04 mg.; Diet 310, 3.01 mg.; Diet 273, 3.15 mg.; Diet 275, 2.80 mg.; and Diet 318, 3.72 mg.*

The factors responsible for the cariogenic action of these diets are highly speculative. Although the unsupplemented control diets are very low in calcium, previous data have shown that the gross percentages of ash, calcium, and phosphorus of both the molar and incisor enamel and dentine remain normal.[1] Previous evidence does point to the possibility that one factor may be a lysine

*We are indebted to Mr. Howard Bakerman of the National Institute of Arthritis and Metabolic Diseases for these analyses.

deficiency.[1, 4] It is of interest that the heat-processed diet for Experiments 318-322 contained a little less than 10.0 per cent free sugar in the form of lactose. Essentially smooth surface caries is produced by these diets and it occurs predominantly on the lower molar teeth.

Started at approximately 30 to 35 grams in weight, littermate weanling rats of the Sprague-Dawley strain from the National Institutes of Health colony were housed, 2 per cage, and continued on experiment for 60 days under a regimen of ad libitum eating and drinking. In Experiments 301 and 311 (Table II), 1 c.c. of a solution of Na_2HPO_4—53 mg. phosphorus, was intubated twice daily for 5 days, once on the sixth day of each week. At the same time, control rats in Experiment 310 received 1 c.c. of distilled water by intubation.

In Experiments 274 and 276, the rats were intubated 5 days each week as follows: in mid-morning with 1 c.c. of a solution of $CaCl_2$ containing 50 mg. of Ca and in mid-afternoon with 1 c.c. of a solution containing 114 mg. Na_2HPO_4—25 mg. phosphorus. These intubated solutions approximately equalized the average daily quantities of calcium and phosphorus ingested by the control rats (Exper. 273 and 275) and the pair-mate intubated rats (Exper. 275 and 276). After sacrifice, the dental caries was diagnosed as previously described.[2]

RESULTS AND DISCUSSION

A very striking cariostatic effect of Na_2HPO_4, as in previous studies,[1, 2] became apparent in these experiments. The effect was particularly evident with 1.5 per cent (Exper. 298) and 2.0 per cent (Exper. 303) Na_2HPO_4 in the diet. These two basal diets differed only in per cent of calcium (Table I).

In Experiments 273, 274, 275, and 276, there were no littermate controls which would be necessary to evaluate directly the effect of Na_2HPO_4. However, by comparison with other control groups (297, 302, 318), as well as by comparison with those Na_2HPO_4 intubated (Exper. 274 and 276), there was a low caries incidence (47.5 and 64.1 per cent), about one third less carious teeth, and much lower caries severity scores due to the addition to these diets of 0.40 per cent Na_2HPO_4, together with 1.25 per cent $CaCO_3$. In view of the evidence that $CaCO_3$ had no pronounced cariostatic property in this type of diet,[1] this cariostatic result seems to be due most likely to the presence of 0.40 per cent Na_2HPO_4.

Experiments 299 and 305 brought out no cariostatic effect of $CaHPO_4$ alone, and this result agrees with previous evidence.[1, 2] However, with NaCl in the diet (Exper. 306) significantly less caries resulted.

In Experiment 300, in which the diet contained 1.5 per cent "$Ca_3(PO_4)_2$," there is the suggestion of an increase in caries; certainly no reduction in caries was evident from this highly insoluble phosphate supplement.

Intubated Na_2HPO_4 in Experiments 301 and 311 appears to have reduced the dental caries to an appreciable extent. This reduction does not compare,

however, in either incidence or reduced severity with the reduction due to dietary Na_2HPO_4. A comparison between dietary and intubated Na_2HPO_4, shown by Experiments 273 vs. 274 and 275 vs. 276, brought out a striking difference between the two routes of administered Na_2HPO_4.

While the mode of action of Na_2HPO_4 still is problematical, it has been proposed in a previous publication[1] that this action may be localized in the oral cavity. A relation of NaCl to the action of $CaHPO_4$ shown by Experiments 302, 304, 305, and 306, and its relation to the cariostatic effect of $Ca(H_2PO_4)_2 \cdot H_2O$, shown in Experiments 318, 319, and 320, would appear to support this hypothesis. It seems likely that $CaHPO_4$ especially, and perhaps $Ca(H_2PO_4)_2 \cdot H_2O$, could be solubilized by NaCl, resulting in an increased

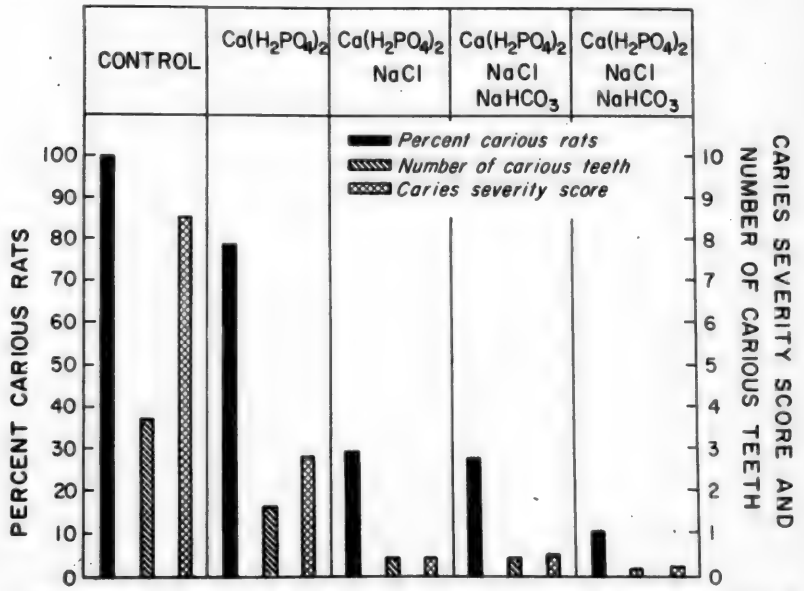

Fig. 1.—The inhibitory effect of a diet supplement of 1.75 per cent $Ca(H_2PO_4)_2 \cdot H_2O$ on experimental caries in white rats.

availability of calcium and phosphate ions within the oral cavity. As was suggested, this could be the cause of the cariostatic effect of $CaHPO_4$ when added to bread which contained NaCl.[2] Although a cariostatic effect of intubated Na_2HPO_4 may throw some doubt on this hypothesis, it may be that the saliva or other oral factors are being influenced by intubated phosphate. It is obvious that intubation did not simulate a diet regimen in the matter of frequency of ingestion of phosphate mineral.

As shown by Experiments 318-322 at a level of 1.75 per cent in the diet, $Ca(H_2PO_4)_2 \cdot H_2O$ caused a marked reduction in caries in four test groups of rats. The addition of 2.25 per cent NaCl increased considerably this cariostatic effect, particularly as regards severity (Exper. 320). The presence of 1.37 per cent $NaHCO_3$ had no apparent further influence on the effect with

$Ca(H_2PO_4)_2 \cdot H_2O$ + NaCl (Exper. 321 and 322). Autoclaving the minerals with the lactose-flour mix also apparently had no effect. A remarkably low incidence (10.2 per cent) and a very low severity score of 0.18 occurred in Experiment 322. Fig. 1 presents these data graphically.

The results of these studies which are particularly interesting are, first, the striking inhibition of caries caused by the addition of $Ca(H_2PO_4)_2 \cdot H_2O$ at a level of 1.75 per cent to the diet. This monobasic phosphate was cariostatic in the diet without added NaCl. It thus appears to be a more effective phosphate than $CaHPO_4$ which, to become cariostatic, required the presence of NaCl in the diet. The effectiveness of $Ca(H_2PO_4)_2 \cdot H_2O$ may be enhanced by the fact that because of rapid solubility both calcium and phosphate ions are readily available in the oral cavity. Second, it is of much interest that $Ca_3(PO_4)_2$* had no cariostatic action. Thus, the striking inactivity of this phosphate supports the suggestion that rapid solubility in the oral cavity is a determining factor for a cariostatic action of these minerals. Further work will be required, however, to verify this hypothesis. The result does emphasize however that a calcium or phosphorus supplement per se is not necessarily cariostatic. Finally, the results with $Ca(H_2PO_4)_2 \cdot H_2O$ are interesting and provocative because of the presence of the anhydrous form of this compound in self-rising flours and other prepared mixes which are a part of the human dietary. The cariostatic effect of these commercial phosphated mixes as used in the human dietary is being studied.

SUMMARY

1. A diet containing whole wheat flour and lactose autoclaved, and liver powder, produced caries to a significant extent in white rats.

2. Dietary supplements of Na_2HPO_4 and $Ca(H_2PO_4)_2 \cdot H_2O$ were highly cariostatic; precipitated basic calcium phosphate and $CaHPO_4$ were not cariostatic. The addition of NaCl to the diet was instrumental in rendering $CaHPO_4$ cariostatic and improved the cariostatic effect of $Ca(H_2PO_4)_2 \cdot H_2O$.

3. When administered by intubation, Na_2HPO_4 was considerably less cariostatic than when ingested in the diet.

4. The results suggest that relative solubility in the oral cavity was a factor in the cariostatic action of the phosphates studied.

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*Precipitated basic calcium phosphate.

THE EVANSTON DENTAL CARIES STUDY

XIX. Prevalence of Malocclusion of Children in a Fluoridated and Control Area

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DENTAL DECAY is considered by some to exert a detrimental effect on occlusion; however, there is no complete agreement in the literature establishing the amount of dental malocclusion stemming from the early loss of tooth material. Plater[1] reported the range to be from 18 to 35 per cent, depending on who made the classification. Where there is a pronounced reduction in the incidence of dental caries among school children it is, therefore, logical to assume there might also be a reduction in the prevalence of malocclusion.

The Evanston children have shown a remarkable lowering of dental caries rates since the Evanston water was fluoridated.[2] The rates of the control, fluoride-free area, children have remained practically unchanged over a 9-year period.[2] The two groups with dissimilar caries rates provided a reliable basis for comparing the incidence of malocclusion among children with high caries rates with those having low caries rates.

The dental records provide excellent information concerning the premature loss of the second deciduous molar teeth with respect to occlusion. Many of the same Evanston children examined at 6, 7, and 8 years of age were examined again at 12, 13, and 14 years of age. This made possible the opportunity to observe the occlusion of these children during a growth period.

REVIEW OF LITERATURE

Newman[3] stated that malocclusion of the teeth was an unsolved dental public health problem in most American communities. Surveys[3-14] among various age groups from 2 through 18 years of age showed malocclusion to be present from a low of 36 per cent[9] to a high of 78 per cent[14] of the cases with the majority in the 44 to 60 per cent bracket.[3, 5-10, 12] In a survey of Negro children, 12 to 16 years of age, Altemus[15] reported 83 per cent with malocclusion. Foster,[16] in comparing malocclusion of white and Indian children (no age listed), reported 49 and 46.3 per cent, respectively. Davies[17] reported the incidence of malocclusion among the Polynesians of Pukapuka

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(Danger Island) to be 35.6 per cent. The group ranged in age from 5 to 70 years. Huber and Reynolds[18] reported on 500 male college students, 16 to 36 years of age, and found 91.4 per cent having some degree of malocclusion.

Plater,[1] Adler,[19] Massler,[20] Silver,[12] Sannerud,[21] and Lundstrom[22] pointed to the adverse effect of the loss of tooth structure on occlusion. Pelton and Elsasser[23] in their study at Coeur d'Alene, Idaho (fluorine—none), and Nampa, Idaho (fluorine—1.5 ppm), where Coeur d'Alene had a substantially higher caries rate than Nampa, found their data did not support the hypothesis which regards dental caries as a primary etiologic factor in malocclusion. McCall[9] found only a small number of children (80 out of 775) in whom the malocclusion was directly related to premature extractions. He stated, nevertheless, that a high percentage of malocclusion of various types was found in those who had experienced such extractions. In the 2- to 6-year-old children who had premature extractions, 52 per cent showed some type of malocclusion as contrasted with 32 per cent with malocclusion who had no premature extractions. Davies[17] found only a few cases where the premature loss of deciduous teeth, prolonged retention of deciduous teeth, and the abnormal habits were found to be contributing causes of malocclusion.

METHOD

The mechanism of the Evanston Dental Caries Study has been previously reported by Blayney and Tucker.[24] In this study the public school white children were classified according to age, year of examination, exposure to fluoridated water, type of occlusion, premature loss of second deciduous molar teeth prior to examination at 6, 7, or 8 years of age, and active and previous orthodontic treatment. No distinction was made between male and female as Massler and Frankel,[14] Newman,[3] and Davies[17] found no statistical difference in the incidence of malocclusion between boys and girls.

The classification of malocclusion was judged primarily from the positions mesiodistally assumed by the first permanent molars on their erupting and locking. In cases marked as normal occlusion, the molars were in proper mesiodistal relationship, and the remaining teeth were in normal relationship.

Class I Malocclusion.—These cases had a normal mesiodistal relationship to the first molars. However, one or more of the molars may have been in buccoversion or linguoversion. Cases of wide spacing, anterior teeth rotated, or inclined to the labial or lingual sides, were considered as Class I malocclusion. Prior to the eruption of the first permanent molar, classification was judged by dental arch relationship. When one or more of the second deciduous molars had been lost prematurely and the first permanent molar or molars had drifted forward, the classification was based upon the arch relationship.

Class II Malocclusion.—These were cases in which the lower first permanent molars were in disto-occlusion to an amount of more than one half the width of one cusp on each side. When this condition existed on one side only, the classification II—subdivision was used with the explanation "one side only" or "right side only." All such cases were grouped as Class II.

Division I: These were cases in which the maxillary incisors were in labioversion.

Division II: These were cases in which the maxillary incisors were in linguoversion or torsoversion (turned or rotated).

Class III Malocclusion.—These were cases in which the lower molars were in mesial relationship to the upper molars to an amount of more than one half the width of one cusp. Where this condition existed on one side only, the classification III—subdivision was used with the explanation "one side only" or "right side only."

Cases in Which Characteristics Were Not Well Defined.—The anatomic factors and the inclined planes were studied in an attempt to determine the type of malocclusion the case had developed and how it might be classified. Malocclusion of the deciduous teeth, especially cases where the first permanent molar had not erupted, was determined by dental arch relationship and anatomic relationships. Cases in which one or more teeth had been lost were classified on the basis of the closest or nearest classification fitting the condition and type of occlusion prevailing before the tooth or teeth were lost.

Although none of the examining dentists in the Evanston Dental Caries Study are trained orthodontists, they have schooled themselves regarding some of the problems in recognizing various types of malocclusion and have had additional experience in the recognition of carious lesions. Even so, some cases were extremely puzzling and difficult to classify. After a consultation, if no definite type of malocclusion was readily recognized, the case was placed in that class of malocclusion whose description most nearly fitted the particular condition.

FINDINGS

When the 6- to 8-year-old children were separated according to year of examination, the 1946 pre-fluoride base line group showed 62.49 per cent with normal occlusion while the 1955 group, with 8 years' exposure to fluoridated water, had 70.46 per cent with normal occlusion (Table 1). This improvement seen in the occlusion of the fluoride area children is more pronounced when it is noted that the fluoride-free control area children showed little difference between examinations spaced 9 years apart. The figures in the control area would hardly be expected to be the same over a 9-year period, nevertheless the results were not far apart (72.28 per cent in 1947 and 69.02 per cent in 1956). There was a greater difference between the 1946 and 1955 figures than would normally be expected.

When the 12-, 13-, and 14-year-old children examined in the 1946 pre-fluoride base line group were compared with those examined in 1957 after a 10-year exposure to fluoridated water, the pre-fluoride group showed 44.17 per cent with normal occlusion while the fluoride group of 1957 had 53.68 per cent with normal occlusion (Table I). In the fluoride-free control area, the results of the 1947 and 1956 examinations were strikingly close. The 1947

TABLE I

WHITE CHILDREN. PERCENTAGE OF CHILDREN WITH NORMAL AND ABNORMAL OCCLUSION IN THE PRE-FLUORIDE AND FLUORIDE GROUP IN EVANSTON, ILL., AND THE CONTROL FLUORIDE-FREE GROUP, OAK PARK, ILL.

YEAR	AGE (YEARS)	NUMBER OF CHILDREN EXAMINED	NUMBER OF CHILDREN WITH NORMAL OCCLUSION	NUMBER OF CHILDREN WITH MALOCCLUSION
		Evanston, Ill.		
1946 (Pre-fluoride base line)	6-8	1,397	873 (62.49%)	524 (37.51%)
1955 (8 years of fluoridation)	6-8	789	556 (70.46%)	233 (29.54%)
1946 (Pre-fluoride base line)	12-14	1,227	542 (44.17%)	685 (55.83%)
1957 10 years of fluoridation	12-14	706	379 (53.68%)	327 (46.32%)
		Oak Park, Ill. (control fluoride-free area)		
1947	6-8	1,032	746 (72.28%)	286 (27.72%)
1956	6-8	1,133	782 (69.02%)	351 (30.98%)
1947	12-14	1,022	561 (54.89%)	461 (45.11%)
1956	12-14	1,182	607 (51.35%)	575 (48.65%)

group had 54.89 per cent with normal occlusion while 51.35 per cent of the 1956 group had normal occlusion. It is of interest to note that the differences between the control area examinations in 1947 and 1956 for the 6- to 8-year-olds and the 12- to 14-year-olds were of about the same magnitude: 3.26 percentage points for the 6- to 8-year-olds and 3.54 percentage points for the 12- to 14-years-olds.

When the percentage of cases in each classification of malocclusion, Classes I, II and III, was considered, it was seen (Table II) that the number of cases with Class I malocclusion in 1955 and in 1957 was considerably less than in 1946. The percentage differences in the number of Class II cases between 1946 and 1955-1957 varied with minor excursions for the children 7, 8, 12, and 14 years old. The 2.70 per cent with Class II malocclusion among the 6-year-olds in 1946 rose to 8.42 per cent in 1955. The 13-year-olds in 1946 with 16.53 per cent dropped to 11.64 per cent in 1957. The percentage of cases with Class III malocclusion fluctuated for the 6 ages (6, 7, 8, 12, 13, 14) between 1946 and 1955-1957. Although there were very few cases in this category (Class III), only the 8-year-old children in 1955 had an increase in the percentage of cases falling in Class III. In the control area (Table III), the widest range between the 1947 and 1956 examinations was found within the group of 12-year-old children. In 1947, 27.05 per cent had Class I malocclusion; in 1956, 36.54 per cent. The 13-year-old children had a considerable difference in the number of Class I cases between the two examinations, as 25.99 per cent of the 1947 group and 35.21 per cent of the 1956 group had Class I malocclusion.

TABLE II
WHITE CHILDREN. TYPE OF OCCLUSION. EVANSTON, ILL. (FLUORIDE AREA)

AGE	NORMAL		CLASS I		CLASS II		CLASS III		TOTAL CHILDREN EXAMINED
	NO.	PER CENT	NO.	PER CENT	NO.	PER CENT	NO.	PER CENT	
Year, 1946—Base Line Pre-fluoride									
6	265	79.58	56	16.82	9	2.70	3	0.90	333
7	347	66.10	124	23.62	46	8.76	8	1.52	525
8	261	48.43	205	38.03	66	12.24	7	1.30	539
12	159	54.27	95	32.42	35	11.95	4	1.36	293
13	194	40.08	201	41.53	80	16.53	9	1.86	484
14	189	42.00	188	41.78	61	13.56	12	2.66	450
Year, 1955—8 Years of Fluoridation									
6	250	84.18	20	6.73*	25	8.42	2	0.67	297
7	173	71.19	49	20.16	19	7.82	2	0.83	243
8	133	53.41	71	28.52*	37	14.86	8	3.21	249
Year, 1957—10 Years of Fluoridation									
12	114	57.58	57	28.79†	25	12.62	2	1.01	198
13	120	51.72†	82	35.34†	27	11.64	3	1.30	232
14	145	52.54†	91	32.97†	34	12.32	6	2.17	276

*Difference between rate in 1955 and 1946 is statistically significant.
†Difference between rate in 1957 and 1946 is statistically significant.

TABLE III
WHITE CHILDREN. TYPE OF OCCLUSION. OAK PARK, ILL. (CONTROL FLUORIDE-FREE AREA)

AGE	NORMAL		CLASS I		CLASS II		CLASS III		TOTAL CHILDREN EXAMINED
	NO.	PER CENT	NO.	PER CENT	NO.	PER CENT	NO.	PER CENT	
Year, 1947									
6	262	90.03	14	4.81	13	4.47	2	0.69	291
7	288	75.79	62	16.32	26	6.84	4	1.05	380
8	196	54.29	126	34.90	34	9.42	5	1.39	361
12	165	58.72	76	27.05	39	13.88	1	0.35	281
13	237	58.66	105	25.99	53	13.12	9	2.23	404
14	159	47.18	119	35.31	49	14.54	10	2.97	337
Year, 1956									
6	340	83.95	40	9.88	21	5.19	4	0.98	405
7	251	71.31	63	17.90	35	9.94	3	0.85	352
8	191	50.80	120	31.91	63	16.76	2	0.53	376
12	170	46.70	133	36.54	61	16.76	—	—	364
13	218	51.17	150	35.21	54	12.68	4	0.94	426
14	219	55.87	127	32.40	42	10.71	4	1.02	392

In determining the percentage of children with normal and abnormal occlusion by separate age (6, 7, 8, 12, 13 and 14 years) for the pre-fluoride base line group, the fluoride group, and the control area examinations of 1947 and 1956, it was apparent that the fluoride group had definite increases in the number of children with normal occlusion when compared to the pre-fluoride base line group for all ages (Tables IV and V). When the control fluoride-free area examinations of 1956 were compared with the 1947 examinations, all ages in 1956 except the 14-year-olds had fewer cases with normal occlusion.

In view of the increase in the number of cases of normal occlusion after fluoridation, we felt it pertinent to determine the prevalence of active or previous orthodontic treatment. Figures for the years 1946, 1947, 1955, 1956,

TABLE IV

WHITE CHILDREN. PERCENTAGE OF CHILDREN, 6, 7, OR 8 YEARS OF AGE, WITH NORMAL AND ABNORMAL OCCLUSION, EVANSTON, ILL. (FLUORIDE AREA) AND OAK PARK, ILL. (CONTROL FLUORIDE-FREE AREA)

AREA—YEAR	TOTAL NUMBER CHILDREN BY AGE	TYPE OF OCCLUSION	AGE IN YEARS (PER CENT) 6	7	8
Evanston base line (pre-fluoride) 1946	6 — 333 7 — 525 8 — 539 1,397	Normal	79.58	66.10	48.43
		Malocclusion	20.42	33.90	51.57
Control (fluoride-free) 1947	6 — 291 7 — 380 8 — 361 1,032	Normal	90.03	75.79	54.29
		Malocclusion	9.97	24.21	45.71
Evanston (8 years of fluoridation) 1955	6 — 297 7 — 243 8 — 249 789	Normal	84.18	71.19	53.41
		Malocclusion	15.82	28.81	46.59
Control (fluoride-free) 1956	6 — 405 7 — 352 8 — 376 1,133	Normal	83.95	71.31	50.80
		Malocclusion	16.05	28.69	49.20

TABLE V

WHITE CHILDREN. PERCENTAGE OF CHILDREN, 12, 13, OR 14 YEARS OF AGE, WITH NORMAL AND ABNORMAL OCCLUSION, EVANSTON, ILL. (FLUORIDE AREA) AND OAK PARK, ILL. (CONTROL FLUORIDE-FREE AREA)

AREA—YEAR	TOTAL NUMBER CHILDREN BY AGE	TYPE OF OCCLUSION	AGE IN YEARS (PER CENT) 12	13	14
Evanston base line (pre-fluoride) 1946	12 — 293 13 — 484 14 — 450 1,227	Normal	54.27	40.08	42.00
		Malocclusion	45.73	59.92	58.00
Control (fluoride-free) 1947	12 — 281 13 — 404 14 — 337 1,022	Normal	58.72	58.66	47.18
		Malocclusion	41.28	41.34	52.82
Control (fluoride-free) 1956	12 — 364 13 — 426 14 — 392 1,182	Normal	46.70	51.17	55.87
		Malocclusion	53.30	48.83	44.13
Evanston (10 years of fluoridation) 1957	12 — 198 13 — 232 14 — 276 706	Normal	57.58	51.72*	52.54*
		Malocclusion	42.42	48.28	47.46

*Difference between rate in 1957 and 1946 is statistically significant.

and 1957 are shown in Table VI. There was very little difference between the number of children with treatment in 1946 and the fluoride group of 1955 and 1957. In the control area, children examined in 1956 showed a slight increase in the number of those treated when compared with the treated ones of 1947.

TABLE VI

WHITE CHILDREN. ORTHODONTIC TREATMENT (PREVIOUS AND ACTIVE), EVANSTON, ILL. (FLUORIDE AREA) AND OAK PARK, ILL. (CONTROL FLUORIDE-FREE AREA)

AREA—YEAR	AGE IN YEARS	NUMBER CHILDREN EXAMINED	NUMBER WITH TREATMENT	PER CENT
Evanston base line (pre-fluoride), 1946	6-8 and 12-14	2,624	196	7.47
Evanston fluoride group, 1955 and 1957 examinations	6-8 and 12-14	1,495	119	7.96
Control (fluoride-free) 1947 examinations	6-8 and 12-14	2,054	114	5.55
1956 examinations	6-8 and 12-14	2,315	139	6.00

The Premature Loss of the Second Deciduous Molar.—Since the dental records of the Evanston Dental Caries Study provide excellent information concerning the premature loss of the second deciduous molar tooth, an effort was made to compare the occlusion of the children with the premature loss of one or more second deciduous molar teeth with those children with no such loss.

TABLE VII

WHITE CHILDREN. CASES WITH AND WITHOUT PREMATURE* LOSS OF SECOND DECIDUOUS MOLAR TEETH (UPPER, LOWER OR BOTH) AND TYPE OF OCCLUSION. CASES WITH SPACE MAINTAINERS, ACTIVE AND PREVIOUS ORTHODONTIC TREATMENT ELIMINATED. EVANSTON, ILL. (FLUORIDE AREA) AND OAK PARK, ILL. (CONTROL FLUORIDE-FREE AREA)

AREA—YEAR	AGE	TOTAL NUMBER CHILDREN	WITHOUT LOSS SECOND DECIDUOUS MOLAR (NUMBER CHILDREN)		WITH LOSS SECOND DECIDUOUS MOLAR (NUMBER CHILDREN)	
			NORMAL	MALOCCLUSION	NORMAL	MALOCCLUSION
Evanston, 1946 (pre-fluoride)	6-8	1,386	828 (63.69%)	472 (36.31%)	39 (45.34%)	47 (54.65%)
Evanston, 1955 8 yrs. of fluoridation)	6-8	786	550 (70.78%)	227 (29.22%)	6 (66.67%)	3 (33.33%)
Control, 1947 (fluoride-free)	6-8	1,014	695 (74.01%)	244 (25.99%)	41 (54.67%)	34 (45.33%)
Control, 1956 (fluoride-free)	6-8	1,126	723 (69.78%)	313 (30.22%)	55 (61.11%)	35 (38.89%)
Totals		4,312	2,796 (69.00%)	1,256 (31.00%)	141 (54.23%)	119 (45.77%)

*Second deciduous molar lost prior to examination at 6, 7, or 8 years of age.

Those children with a history of previous or active orthodontic treatment, as well as children with space maintainers, were eliminated. The loss of the second deciduous molar took place prior to the examination at 6, 7, or 8 years of age. In 1946, 1,386 children, 6, 7, and 8 years of age, were examined (Table VII). Eighty-six of these children experienced the premature loss of one or more second deciduous molar teeth. In 1947, 54.67 per cent had no malocclusion; the figure rose to 66.11 per cent in 1956. Of the 6-, 7-, and 8-year-old children, examined in 1946, 1947, 1955, and 1956, with premature loss of the second deciduous molar teeth, 141 (54.23 per cent) had normal occlusion while

119, or 45.77 per cent, had malocclusion. It was also noted that of the 4,052 children examined with no loss of the second deciduous molar tooth at 6, 7, or 8 years of age, 2,796 (69.0 per cent) had normal occlusion while 1,256 (31.0 per cent) had malocclusion.

When the 86 children with premature loss of the second deciduous molar tooth, examined in 1946, were studied according to respective age, it was apparent that the effect upon occlusion was most noticeable at 8 years of age. In the fluoride area group and the control group (Tables VIII and IX), there was a pronounced increase in the number of cases of malocclusion as the age increased from 6 to 8.

TABLE VIII

WHITE CHILDREN. CASES WITH PREMATURE* LOSS OF SECOND DECIDUOUS MOLAR TEETH (UPPER, LOWER OR BOTH) AND TYPE OF OCCLUSION. CASES WITH SPACE MAINTAINERS, ACTIVE AND PREVIOUS ORTHODONTIC TREATMENT ELIMINATED. EVANSTON, ILL. (FLUORIDE AREA)

AGE	NUMBER OF CASES WITH PREMATURE LOSS SECOND DECIDUOUS MOLAR	TYPE OF OCCLUSION			
		NORMAL	PER CENT	MALOCCLUSION	PER CENT
1946 (pre-fluoride group)					
6	10	9	90.00	1	10.00
7	23	13	56.52	10	43.48
8	53	17	32.08	36	67.92
Totals	86	39	(45.35)	47	(54.65)
1955 (8 years of fluoridation)					
6	0	—	—	—	—
7	6	4	66.67	2	33.33
8	3	2	66.67	1	33.33
Totals	9	6	66.67	3	33.33

*Second deciduous molar lost prior to examination at 6, 7, or 8 years of age.

TABLE IX

WHITE CHILDREN. CASES WITH PREMATURE* LOSS OF SECOND DECIDUOUS MOLAR TEETH (UPPER, LOWER OR BOTH) AND TYPE OF OCCLUSION. CASES WITH SPACE MAINTAINERS, ACTIVE AND PREVIOUS ORTHODONTIC TREATMENT ELIMINATED. OAK PARK, ILL. (CONTROL FLUORIDE-FREE AREA)

AGE	NUMBER OF CASES WITH PREMATURE LOSS SECOND DECIDUOUS MOLAR	TYPE OF OCCLUSION			
		NORMAL	PER CENT	MALOCCLUSION	PER CENT
1947, Control (fluoride-free group)					
6	13	10	76.92	3	23.08
7	28	17	60.71	11	39.29
8	34	14	41.17	20	58.83
Totals	75	41	(54.67)	34	(45.33)
1956, Control (fluoride-free group)					
6	14	13	92.85	1	7.15
7	35	21	60.00	14	40.00
8	41	21	51.22	20	48.78
Totals	90	55	(61.11)	35	(38.89)

*Second deciduous molar lost prior to examination at 6, 7, or 8 years of age.

The effect on occlusion caused by the premature loss of the second deciduous molar tooth was not as pronounced at age 6 or 7 as it was at age 8, but what effect could this loss be expected to have on the occlusion of a child when he is 12, 13, or 14 years of age? Eighty-four children examined as 6-, 7-, and 8-year-olds, all with the premature loss of the second deciduous molar prior to 6, 7, or 8 years of age, were examined again 6 years later at 12, 13, or 14 years of age. These children had no previous or active orthodontic treatment or the advantage of space maintainers. As shown in Table X, the 8-year-olds, contrasted with the 6- and 7-year-olds, had the lowest percentage of normal occlusion. When examined 6 years later at 14 years of age, these 8-year-olds had the greatest increase in the percentage of normal cases. Among the 6- and 7-year-old children, there were fewer normal occlusion cases when examined at the ages of 12 and 13.

TABLE X

EVANSTON, ILL. (FLUORIDE AREA). EIGHTY-FOUR WHITE CHILDREN, 6 TO 8 YEARS OF AGE, WITH PREMATURE* LOSS OF SECOND DECIDUOUS MOLAR TEETH (UPPER, LOWER OR BOTH) AND TYPE OF OCCLUSION. CASES WITH SPACE MAINTAINERS, ACTIVE AND PREVIOUS ORTHODONTIC TREATMENT ELIMINATED (FIRST EXAMINATION AT 6, 7, AND 8 YEARS OF AGE AND FINAL EXAMINATION AT 12, 13, AND 14 YEARS OF AGE)

	AGE IN YEARS	NUMBER OF CASES	TYPE OF OCCLUSION (NO. OF CASES)	
			NORMAL	MALOCCLUSION
First Examination				
	6	27	22 (81.48%)	5 (18.52%)
	7	28	19 (67.85%)	9 (32.15%)
	8	29	9 (31.03%)	20 (68.97%)
Totals		84	50 (59.52%)	34 (40.48%)
Final Examination				
	12	27	12 (44.44%)	15 (55.56%)
	13	28	14 (50.00%)	14 (50.00%)
	14	29	14 (48.27%)	15 (51.73%)
Totals		84	40 (47.61%)	44 (52.39%)

*Second deciduous molar lost prior to examination at 6, 7, or 8 years of age.

Of 41 children examined at 6 years of age, with no history of premature loss of the second deciduous molar tooth, previous or active orthodontic treatment, or the use of space maintainers, 31 of them, or 75.60 per cent, showed normal occlusion (Table XI). Eight years later, this same group of children had 9 cases (21.95 per cent) of normal occlusion, and 32 cases (78.05 per cent) of malocclusion.

TABLE XI

EVANSTON, ILL. (FLUORIDE AREA). FORTY-ONE WHITE 6-YEAR-OLD CHILDREN WITH NO RECORD OF PREMATURE LOSS OF SECOND DECIDUOUS TEETH AND TYPE OF OCCLUSION. CASES WITH SPACE MAINTAINERS, ACTIVE AND PREVIOUS ORTHODONTIC TREATMENT ELIMINATED (FIRST EXAMINATION AT 6 YEARS OF AGE AND FINAL EXAMINATION AT 14 YEARS OF AGE)

	AGE IN YEARS	NUMBER OF CASES	TYPE OF OCCLUSION (NO. OF CASES)	
			NORMAL	MALOCCLUSION
First examination	6	41	31 (75.60%)	10 (24.40%)
Final examination	14	41	9 (21.95%)	32 (78.05%)

DISCUSSION

In 1955, the rate of dental caries among the 6-, 7-, and 8-year-old children in Evanston, Ill., when compared to that of the base line students, showed a reduction of 62.65 per cent. There was a reduction in dental caries of approximately 46.22 per cent among the 12- to 14-year-olds in the 1957 Evanston group with 10 years' exposure to fluoridation.[26] Plater[1] reported a 30 per cent reduction in the prevalence of malocclusion in the same areas of optimum concentration of 1.0 ppm fluorides. It is interesting to note that the reduction in the incidence of malocclusion among the Evanston children, 6 to 8 years old, after 8 years of fluoridation, was 20.48 per cent, and 17.03 per cent among those 12 to 14 years old after 10 years of fluoridation (Table I). Although this reduction was a decided improvement, it was still somewhat removed from the 30 per cent reduction cited by Plater, especially for the 12- to 14-year group.

The prevalence of malocclusion as presented in this report for the 6-year-olds is somewhat lower than reported by Munblatt[4] and Silver.[12] Approximately 54 per cent of the 6-year-olds as reported by Munblatt had malocclusion (girls 60 per cent and boys 42.8 per cent malocclusion) while Silver[12] reported 61.32 per cent of 6-year-olds with malocclusion. Korkhaus, as reported by Goldstein and Stanton,[10] showed that 43.1 per cent of 643 6-year-old children had malocclusion. Of the 6-year-olds in our report, 20.42 per cent of the 1946 pre-fluoride base line group had malocclusion and 15.82 per cent of the 1955 fluoride group had malocclusion (Table IV).

Goose, Thompson and Winter[6] reported that 69.5 per cent of the 7-year-olds and 53.6 per cent of the 8-year-olds had normal occlusion. Silver[12] reported that 50.95 per cent of the 7-year-olds and 34.05 per cent of the 8-year-olds had normal occlusion. Among the 7- and 8-year-olds in our base line group, 66.10 per cent and 48.43 per cent, respectively, showed normal occlusion (Table IV).

Goose, Thompson, and Winter[6] reported that among children, 12, 13, and 14 years old, 51.9 per cent, 48.9 per cent, and 54.4 per cent, respectively, showed normal occlusion. Silver[12] reported 37.50 per cent, 31.74 per cent, and 30.61 per cent, and the Evanston pre-fluoride 1946 group showed 54.27 per cent 40.08 per cent, and 42.00 per cent, respectively (Table V).

In reporting on the children 6 through 14 years of age, Newman[3] found that approximately 52 per cent of the children had malocclusion. Goose, Thompson and Winter[6] reported that 44 per cent of children 7 to 15 years of age had malocclusion. These figures are somewhat closer to the figure of 46.05 per cent with malocclusion in the combined 6- to 8- and 12- to 14-year-old Evanston 1946 pre-fluoride base line groups (Table XII).

The data in our report quite clearly indicate the need to evaluate the findings on occlusion for the years 1948 through 1954. These additional data tend to reveal the type of change which may be taking place from year to

year. The data for these years have been recorded and placed on punch cards for I.B.M. running but no request has been made to the Bureau of Vital Statistics to tabulate this material.

TABLE XII

WHITE CHILDREN. PERCENTAGE OF CASES IN EACH CLASSIFICATION OF TYPE OF OCCLUSION (CHILDREN 6 TO 8 AND 12 TO 14 YEARS OF AGE). EVANSTON, ILL. (FLUORIDE AREA) AND OAK PARK, ILL. (CONTROL FLUORIDE-FREE AREA)

AREA	YEAR	FLUORIDE	OCCLUSION CLASSIFICATION (PER CENT)			
			NORMAL	CLASS I	CLASS II	CLASS III
Evanston, Ill.	1946	Pre-fluoride	53.95	33.11	11.31	1.63
Evanston, Ill.	1955	8-10 yr. fluoridation	62.56	24.74	11.17	1.53
Evanston, Ill.	1957					
Oak Park, Ill.	1947	None	63.65	24.44	10.41	1.50
Oak Park, Ill.	1956	None	60.01	27.34	11.92	0.73

The effect on malocclusion of the premature loss of a second deciduous molar tooth appeared to be somewhat contradictory. In our classification, the children with the premature loss of a second deciduous molar tooth showed a greater percentage of malocclusion than the group with no such loss. The fact remains that approximately ½ to ⅔ of the children with premature loss of this tooth had normal occlusion. This indicates that, as a contributing factor in the etiology of malocclusion, premature loss apparently was active in approximately 50 per cent or less of the cases of malocclusion. Kronfeld[27] reported that in 29 cases in which a second deciduous molar was lost prematurely there was a loss of space in 20, or 69.71 per cent, of the cases. This is similar to the findings of Silver[12] who reported that in 22 out of 28 (78.6 per cent) of the cases the space was lost and in 6 out of 28 (21.4 per cent) the space was maintained. In our study (Table X), approximately one half of the 29 8-year-old children with loss of a second deciduous molar prior to 6, 7, or 8 years of age, had normal occlusion at 14 years of age. This fact appears to substantiate the finding of Lundstrom[22] that the early loss of deciduous teeth apparently had no influence of a general nature on the development of the dentition, and Davies' report[17] that this loss was a contributing cause in only a small proportion of cases. Sannerud[21] indicated that premature loss of the deciduous teeth was one of the factors causing malocclusion in the permanent dentition, and Ungar[28] stated that the loss of the second deciduous molar caused the highest percentage of impactions and crowding of the succeeding permanent tooth, with the first deciduous molar coming next in this regard.

SUMMARY

The prevalence of malocclusion among children, 6 to 8 and 12 to 14 years of age, is reported. The examinations were separated into pre-fluoride, fluoride, and fluoride-free control groups. The prevalence of malocclusion occurring among the 6- to 8-year-olds after 8 years of fluoridation was 20.48 per cent lower than the pre-fluoride base line group of 6- to 8-year-old children. The 12- to 14-year-old group, after 10 years of fluoridation, showed a decrease of 17.03 per cent when compared with the pre-fluoride base line group of the

same age. The prevalence of malocclusion in the fluoride area in 1955 and 1957 was nearly the same as that found in the 1956 control area examinations. In contrast, however, to the reduced prevalence of malocclusion in the study area group, the control area group showed an increase of 11.76 per cent for the 6- to 8-year-old children and 7.84 per cent for the 12- to 14-year-olds over a 9-year period between examinations.

Of 141 children, 6 to 8 years old, with premature loss of the second deciduous molar tooth, approximately one-half, or 54.23 per cent, had normal occlusion. An examination of 84 children at 6, 7, and 8 years of age with the loss of the second deciduous molar prior to 6, 7, or 8 years of age, revealed 50 cases, or 59.52 per cent, of the group to have normal occlusion. Six years later, 40 cases, or 47.61 per cent, of these same children at 12, 13, and 14 years of age had normal occlusion. These children had neither orthodontic treatment nor the advantage of space maintainers.

No increase was found in the percentage of children having orthodontic treatment for the pre-fluoride and fluoride groups. This percentage showed a slight increase for the control area group in 1956 over the 1947 control area group.

From our observations of the data available in our study at the present time, we feel that no definitive statement can be made regarding fluoridation and its effect on the prevalence of malocclusion.

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THE EFFECT OF ANTIHISTAMINICS ON DENTAL CARIES IN THE RAT

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THE USE of antihistaminic drugs for patients with allergies and related conditions is steadily increasing and it is not uncommon for patients to use such medicinal preparations for prolonged periods of time. While it would be desirable that a minimum of adverse effects occurs as a result of use of these drugs, occasional disturbances are seen. Noteworthy among these, from the dental viewpoint, is the complaint of xerostomia. This clinical finding has been confirmed experimentally by McDonald[1] who has shown that certain antihistaminic drugs do in fact reduce salivary flow in the human being. Since his studies were conducted over very short time periods, it became of interest to determine what effect the chronic usage of different antihistaminics would have on the dental caries experience in rats. These data can then be compared with the effect on dental caries resulting from the partial or total reduction in salivary flow when the salivary glands are surgically removed.

METHOD

The present investigation was divided into two series. In Series I, 98 weanling rats of the Sprague-Dawley strain (60 males and 38 females) were divided into three groups: a control group; Group 2 which received 12.5 mg. per cent tripelennamine citrate added to the animals' drinking water; and Group 3 which received 50 mg. per cent thenyldiamine* added to the drinking water as in Group 2. In order to mask the bitter taste of the antihistaminics, 3 ml. of Sucaryl was added to each liter of the drinking water of all three groups. The animals were maintained on the experiment for 4 months after which time they were sacrificed by chloroform inhalation and the dental caries incidence determined by methods previously described.[2]

In Series II, 163 weanling rats of the same strain (91 males and 72 females) were divided into 4 groups: a control group; Group 2 received chlorothenpyramine; Group 3 received tripelennamine citrate; and Group 4 received thenyldiamine. All antihistaminics were added to the cariogenic diet.† The

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†The composition of the diet used in this study (in per cent) is as follows: yellow corn grits, 52.7; ground yellow whole corn, 11.3; powdered whole milk, 30; alfalfa, 4.8; iodized sodium chloride, 1.0; and irradiated yeast, 0.2.

levels initially used were 0.1 gram per cent, but after 2 weeks this level was increased to 0.2 gram per cent. It remained between these two levels throughout the experimental period of 4 months according to the growth and tolerance of the animals. All of the animals in both series were housed in raised metal cages. The cariogenic diet and distilled drinking water were available ad libitum.

DATA AND DISCUSSION

The results of these studies are found in Table I. These data show that there was a pronounced failure to gain weight in all of the animals in both Series I and II. This was especially noticeable in Series I where both control and the group receiving the antihistaminic drugs failed to gain normally. Since the animals in the control group received no antihistaminic, this failure to gain weight normally must be associated with a failure to eat and drink sufficient food and water as a result of the presence of Sucaryl in the drinking water, although this does not seem too probable due to the established low level of toxicity of this chemical. Evidence suggesting a relationship to the failure to eat and drink sufficient food and the presence of the Sucaryl is substantiated, however, in the weight data found in the animals of Series II in which Sucaryl was not added to the diet. In this series, the control animals gained weight at the rate normally experienced for those receiving this diet. The failure to gain weight occurring in Series I must then be related to the toxicity of the antihistaminics. The most marked failure to gain weight occurred in the animals receiving tripelennamine citrate.

TABLE I

A COMPARISON BETWEEN THE DENTAL CARIES INCIDENCE OF RATS RECEIVING DIFFERENT ANTIHISTAMINICS EITHER IN THE DRINKING WATER (SERIES I) OR THE DIET (SERIES II)

GROUP	NUMBER OF RATS*		WEIGHT GAIN (GRAMS)		MEAN NUMBER OF LESIONS (SEXES COMBINED)	p
	M	F	M	F		
Series I						
None	17	12	177	173	4.9	—
Tripelennamine citrate	9	7	122	86	6.9	0.05
Thenyldiamine	17	9	67	47	5.5	0.1
Series II						
None	17	16	270	161	7.4	—
Chlorothenpyramine	21	16	158	121	7.0	0.5
Tripelennamine citrate	19	12	58	32	6.6	0.1
Thenyldiamine	22	17	154	97	7.8	0.5

*At the termination of the study.

In the second series of studies where the antihistaminic agents were incorporated in the diet, the concentrations used were such that about 15 to 18 mg. of the drugs were consumed each day. Assuming a rat body weight of 150 grams, the animals would receive 100 mg. of the drugs per kilogram per day. This represents about a fiftyfold increase in the recommended dosage for these drugs, thus accounting for the toxic effects which were observed.

The effects of the antihistamines on the incidence of dental caries are also found in Table I. In the animals of Series I the tripelennamine citrate significantly increased the incidence of dental caries. The thenyldiamine also increased the number of lesions, but the difference was not significant. In the animals of Series II, the presence of the antihistaminics had no effect on the dental caries incidence, even though sufficient levels were being ingested to result in definite toxic effects on the animal.

One may feel that the reason why there was less caries in the Series I animals when tripelennamine citrate and thenyldiamine were fed in the drinking water than in the Series II animals when the antihistaminics were placed in the diet may be related to the decreased food consumption in the Series I animals. It is difficult to speculate in this regard, since previous studies have shown that when there is a drastic (40 to 60 per cent) reduction in food intake in the rat there is a significant caries reduction,[3] but when only a moderate food restriction occurs, there is little, if any, modification of the rate of caries formation. From these observations, one must conclude that caries development in the rat is a complex picture and that many of the variables which initiate and extend dental caries are not yet defined.

The results of this study suggest that the use of these antihistaminic drugs for prolonged periods of time is not directly associated with a significant caries increase in the rat, although there is some suggestive evidence that there is a tendency for more caries. More work is needed in this area, especially under conditions where food intake and antihistaminic dosage is more closely controlled. It would also be of interest to correlate the effect of different antihistaminics on salivary flow and viscosity in the rat and the incidence of dental caries.

SUMMARY

The administration of tripelennamine citrate and thenyldiamine at a level of 12.5 mg. per cent and 50 mg. per cent, respectively, in the drinking water of rats receiving a cariogenic diet resulted in significantly more dental caries in the animals receiving tripelennamine citrate but not in the animals receiving thenyldiamine, although a tendency for more caries was found in the latter animals. When chlorothenpyramine, tripelennamine citrate, and thenyldiamine were added to the individual diets of the animals (at levels varying between 0.1 and 0.2 gram per cent) there were no significant differences in dental caries when compared to a control group not receiving antihistaminics. All of the antihistaminics resulted in definite toxic effects to the animals as manifested by failure to gain weight normally.

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STUDIES ON THE ANTIBACTERIAL ACTION OF HUMAN SALIVA

I. A Bactericidin for Lactobacilli

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INTRODUCTION

THE ORAL microbiota is known to include a wide spectrum of bacterial species, many of which are potentially pathogenic. Despite the frequency with which the latter have been isolated, as permanent or transient members of the oral flora, they infrequently cause oral infection. It has been postulated that antibacterial factors present in the saliva may be of major significance in regulating this oral resistance to infectious agents.

Antibacterial action of human mixed saliva has been reported by numerous investigators. In 1934, Dold[1] described substances in saliva which inhibited the growth of a wide spectrum of bacterial species. These he termed "inhibins." More specifically, those which inhibited the growth of the diphtheria bacilli were called "zidins." Thompson and his co-workers,[2, 3] Bethege, Soehring and Tschesche,[4] and Hegemann[5] reported that the in vitro diphthericidal activity of the mixed saliva was due to hydrogen peroxide produced by the oral alpha streptococci. Recently, Bartels, Blechman, and Cavallaro[6] demonstrated that freshly stimulated mixed saliva inhibited the growth of beta hemolytic streptococci. They reported that the antimicrobial action appeared to be associated with organisms of the oral microbiota. These and numerous other reports indicate that bacterial antibiosis plays a role in the resistance of the oral mucosa to infection by its regulating action on the oral flora.

Fleming[7] reported that mixed saliva contains the enzyme, lysozyme, which acts on many species of bacteria through its hydrolyzing action on the mucopolysaccharides of the bacteria cell wall. Hoerman, Englander, and Shklair[8] confirmed these findings with mixed saliva and, in addition, surveyed the lysozyme titers in relatively pure salivary secretions. They found that the lysozyme titers in submaxillolingual saliva were substantially higher than those in parotid saliva.

Clough, Bibby, and Berry[9] used the well plate technic to study the lactobacillus growth-inhibiting property of mixed human saliva obtained from 204 individuals. They found antilactobacillus activity in 250 of the 260 samples

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tested. The active component was found to be bactericidal and nonlytic and, in addition, the centrifuged saliva was as active as uncentrifuged saliva. This activity of the saliva was completely destroyed by heating to 75° C. for 5 minutes. Zeldow[10] confirmed these findings and, in addition, observed that the active principle found by others in mixed saliva was also contained in pure parotid saliva. The results indicated that variations occurred in the concentration of the active principle in parotid and mixed saliva collected from the same subject. The factor appeared to be an endogenous component of the saliva and not of a microbial origin. More recently, Kerr and Wedderburn[11] supported these conclusions. They reported that the component inhibitory to lactobacillus was also found in the secretions of the submaxillary gland. By use of the well plate method, they extended the antibacterial spectrum of parotid and submaxillary saliva to include *Micrococcus pyogenes* var. *aureus*, *Micrococcus pyogenes* var. *albus*, *Streptococcus salivarius*, and *Micrococcus lysodeikticus*, as well as three species of lactobacilli.

Reports correlating antimicrobial salivary factors to caries activity have also appeared in the literature. Hill[12] found a factor to be present in the mixed saliva of caries-resistant individuals which deterred growth of *Lactobacillus acidophilus*. This salivary factor remained active after heating at 80° C. for a period of one hour and after dialysis for 24 hours in repeated changes of distilled water. Green and Dodd[13] reported a heat labile non-dialyzable factor in the saliva of caries-immune human beings which inhibited growth of lactobacilli and streptococci. This substance was found lacking in the saliva of caries-susceptible individuals.

Present evidence indicates that very little is known about the innate defenses of the oral cavity against infection. The postulate that endogenous salivary components play an important part in the unusual resistance of the mouth to infection needs further substantiation. The present report is the result of further attempts to characterize and quantitate the lactobacillus bactericidin present in parotid and submaxillary saliva.

MATERIALS AND METHODS

Unless specified otherwise, the following materials and procedures were used throughout this investigation.

Assay Organism.—The test organism used was *L. acidophilus*, ATCC 4357. The cultures were maintained at 37° C. on lactobacillus selective (LBS*) slants or broth.[14] Standardized suspensions of the organisms were made by diluting the bacteria obtained from a 24-hour broth culture with distilled water so as to contain approximately 3×10^7 organisms per milliliter. Turbidimetric readings on a Coleman Junior spectrophotometer were compared to a standardized curve prepared from bacterial colony counts.

Saliva.—Since it has been demonstrated that various antibiotics are detected in the saliva after systemic administration,[15, 16, 17] both the parotid and

*Available from Baltimore Biological Laboratories, Baltimore, Md.

submaxillary salivas were collected from human adults who had not previously received antibiotics within a period of 2 months. In both instances, exogenous stimulation by critic acid troches was found superior to that induced by paraffin chewing, since with this procedure the musculature remained relatively relaxed, thereby avoiding the displacement of the apparatus. The procedures of collection were conducted as aseptically as possible, using sterile apparatus and application of 1 per cent tincture of iodine and 70 per cent alcohol to the area. Parotid saliva was collected directly from Stensen's duct according to the method described by Lashley.[18] Pure submaxillary saliva, however, was obtained by cannulation of Wharton's duct to the point at which it makes its inferior turn at the posterior border of the mylohyoid muscle. These technics ensured the collection of pure secretions.[19, 20]

Immediately after collection, bacterial counts of the saliva were made on blood agar plates which were incubated at 37° C. under aerobic and anaerobic conditions. Although sterile saliva was obtained in only a few cases, the bacterial count of the remaining samples did not exceed 1 per cent of the normal bacterial count of the mixed saliva. All specimens were then stored at –30° C. until used.

Since colony counts indicated that the saliva exerted a marked killing action on the assay organism under the experimental conditions used, the terms "lactobacillus bactericidin" and "antilactobacillus activity" are hereafter used interchangeably to describe this effect.

Quantitative Bactericidin Test.—The lactobacillus bactericidin test was performed in sterile Kahn tubes by first making twofold serial dilutions of the saliva in LBS broth (pH 5.5±) to give final volumes of 0.5 ml.* To each tube was added 0.1 ml. of a standardized suspension of the assay organism. One tube without saliva served as a control. The tubes were incubated at 37° C. for 18 hours and examined for turbidity as evidence of growth. Except for an occasional twofold variance the results were reproducible in most instances.

For quantitative comparison, bactericidal activity is expressed in units. One unit is defined as the minimum amount of bactericidin present in 0.5 ml. of the dilution of saliva which will inhibit the growth of the bacteria contained in the 0.1 ml. of inoculum added. Therefore, if a 1:8 dilution was the highest dilution demonstrating complete visible inhibition, such a saliva would contain 16 units of bactericidin per milliliter.

Concentration.—In order to work with a more potent solution of bactericidin, concentration of the active principle was accomplished by lyophilizing the saliva.[10] For use it was resuspended in sterile distilled water to the desired concentration, usually 4 times the original concentration. Not all constituents of the concentrated saliva were soluble upon the addition of water and the insoluble fraction was removed by centrifugation.

Dialysis.—One volume of saliva was dialyzed against 15 volumes of deionized water at 4° C. for 24 hours in a rotating dialyzer similar to the one

*Control tests with a constant concentration of broth indicated that the variations in the concentration of broth in the different tubes of the standard test did not influence the results.

described by Durrum, Smith, and Jetton.[21] The dialyzed saliva was stored at 4° C. The dialysate was then concentrated by lyophilization and reconstituted with de-ionized water to the original volume of the saliva.

RESULTS

Quantitative Survey of Bactericidin Levels in Human Saliva and Serum.—A comparison of parotid and submaxillary saliva collected from the same individual is shown in Table I. In each of the 25 cases the bactericidin activity ranged from 4 to 64 units per milliliter. Whereas the 2 secretions demonstrated equal titers in 11 of the individuals, in 13 cases the activity of parotid saliva was greater than that of submaxillary saliva. In only a single subject did the submaxillary saliva have a higher titer than parotid saliva.

TABLE I
COMPARISON OF THE BACTERICIDIN ACTIVITY OF PAIRED PAROTID AND SUBMAXILLARY SALIVAS*

	NUMBER OF SUBJECTS	BACTERICIDIN UNITS PER MILLILITER	
		PAROTID	SUBMAXILLARY
P = S†	1	4	4
	2	8	8
	7	16	16
	1	32	32
P > S	3	16	8
	1	32	8
	8	32	16
	1	64	32
P < S	1	8	16

*A sample of parotid saliva and sample of submaxillary saliva were collected simultaneously from each subject.
†P—parotid; S—submaxillary.

The quantity of lactobacillus bactericidin in serum was measured in the same manner. Tests were also conducted on serum using trypticase soy broth (pH 7.3±). No antilactobacillus activity was detected in the serum.

Effect of Lyophilization on the Bactericidin System.—The results in Table II demonstrate that the antilactobacillus activity can be proportionally increased by lyophilization and resuspension in distilled water to a determined concentration. It was found that the soluble fraction (supernate) contained all of the original bactericidin activity.

TABLE II
THE EFFECT OF LYOPHILIZATION ON THE LACTOBACILLUS BACTERICIDIN

PAROTID SALIVA			SUBMAXILLARY SALIVA		
SUBJECT	PREPARATION	BACTERICIDIN (UNITS/ML.)	SUBJECT	PREPARATION	BACTERICIDIN (UNITS/ML.)
A	Unconcentrated	8	A	Unconcentrated	16
	Concentrated*	64		Concentrated*	64
	Supernate	64		Supernate	64
	Sediment	0		Sediment	0
B	Unconcentrated	16	B	Unconcentrated	8
	Concentrated*	64		Concentrated*	32
	Supernate	64		Supernate	32
	Sediment	0		Sediment	0

*Reconstituted with sterile distilled water to one-fourth original volume.

Effect of Dialysis on the Bactericidin System.—The results of dialysis tests presented in Table III show that both the dialyzed saliva and concentrated dialysate, dissolved in de-ionized water, lacked antibacterial activity. It was found, however, that bactericidal activity was re-established when the dialyzed saliva and the concentrated dialysate were recombined in equal volume. Apparently, at least two components are necessary for the functioning of the bactericidin system; namely, a non-dialyzable portion and a component that passed through the cellophane membrane. This latter dialyzable portion apparently contains a cofactor.

TABLE III
THE EFFECT OF DIALYSIS ON THE LACTOBACILLUS BACTERICIDIN

PAROTID SALIVA			SUBMAXILLARY SALIVA		
SUBJECT	PREPARATION	BACTERICIDIN (UNITS/ML.)	SUBJECT	PREPARATION	BACTERICIDIN (UNITS/ML.)
A	Undialyzed	32	A	Undialyzed	16
	Dialyzed	0		Dialyzed	0
	Dialysate	0		Dialysate	0
	Recombined*	32		Recombined*	8
B	Undialyzed	16	B	Undialyzed	16
	Dialyzed	0		Dialyzed	0
	Dialysate	0		Dialysate	0
	Recombined*	16		Recombined*	16

*Following dialysis the material retained by the membrane was recombined with a concentrate prepared from the dialysate.

Heat Stability of the Bactericidin.—The heat stability of the bactericidin system was established by heating aliquots of the secretions of both glands for 5 minutes at 5° C. increments of temperature and varying hydrogen ion concentrations. The behavior of the two salivas was identical. Moreover, the effect of heat on the activity of undialyzed saliva and on the non-dialyzable fraction was the same. Whereas both materials were found to be completely inactivated at 75° C. for 5 minutes at pH values of 3.0 and in the range of 7.0 to 11.0, they were unaffected by such treatment at pH 5.5. The dialysate was found to retain its capacity to reactivate the system after similar heat treatment, irrespective of the hydrogen ion concentration.

Prolonged incubation of the salivas for 48 hours at 37° C. did not alter their antilactobacillus activity.

Effect of Filtration.—Paired, one milliliter aliquots of saliva were passed in sequence through a Swinney filter and the filtrates collected separately. Whereas the second aliquot demonstrated the same bactericidin titer as the unfiltered control, the first aliquot yielded a filtrate with approximately 50 per cent of the original activity.

Trials on Lysozyme and Salivary Amylase.—Crystalline egg white lysozyme in tenfold increments of concentration from 5 to 5,000 γ per milliliter did not inhibit the assay organism. Furthermore, it was found that salivary lysozyme remained active against *Micrococcus lysodeikticus* when heated to 75° C. for 5 minutes at pH 3.0.

Purified amylase,* isolated from pooled human mixed saliva,[22] did not possess antilactobacillus activity.

*Kindly supplied by Dr. E. H. Fischer and Dr. E. A. Stein, Department of Biochemistry, University of Washington, Seattle, Wash.

DISCUSSION

Kerr and Wedderburn[11] have recently called attention to the limitations of the commonly used well plate bacterial inhibition test and emphasized its unreliability for quantitative determination. Preliminary trials conducted in the course of the present investigations have likewise shown that, although the well plate method is adequate for the qualitative determination of lactobacillus bactericidin in saliva, it is unsuitable for quantitative assay. Consequently, a serial dilution method was adopted for the quantitation of the salivary lactobacillus bactericidin. A basic unit of activity was defined so that the results of future investigations can be compared with those described herein.

The lactobacillus bactericidin systems in both parotid and submaxillary saliva appear to be identical. Moreover, the results of the dialysis and heat stability experiments, which showed that a dialyzable heat stable cofactor is necessary for the activity of a less heat stable non-dialyzable component, suggest that the active principle is distinct from the salivary antilactobacillus factors described by Hill[12] and Green and Dodd.[13]

The results of the present study, which showed that pure secretions essentially free of bacteria possess high levels of lactobacillus bactericidin, indicate that the lactobacillus bactericidin is an endogenous product of the salivary glands rather than a product of bacteria of the oral flora. Since, in a previous report,[10] it was also noted that the bactericidin content of pure parotid saliva frequently exceeded that of mixed saliva rich in bacteria, it seems probable that none of the antilactobacillus activity studied results from products of bacterial origin.

Although antibacterial components of endogenous origin have for the most part been described in serum, they may be present in many other tissues. Properdin,[23] β lysin,[24, 25] and Tillett factor[26] serve to exemplify this. Lysozyme[7, 8] has been found to be present in many tissue fluids including serum and saliva. In contrast, the lactobacillus bactericidin present in saliva has not been detected in serum. It is possible that the production of lactobacillus bactericidin is limited to the salivary glands.

SUMMARY

The results further characterize a salivary bactericidin active against *Lactobacillus acidophilus*, ATCC 4357.

The lactobacillus bactericidin was quantitated in paired parotid and submaxillary salivas collected from 25 individuals. Whereas there was marked variation between subjects ranging from 4 to 64 units of bactericidin per milliliter, there was less variation between the paired salivas of the two glands for given subjects. With one exception, the bactericidin levels in the parotid salivas were equal to or greater than those of the submaxillary salivas. Tests for the lactobacillus bactericidin in the serums of the various subjects were negative.

The lactobacillus bactericidin is apparently a salivary factor which requires a dialyzable heat stable cofactor for its activity. It is moderately heat stable over a wide range of pH with maximum stability at pH 5.5.

The lactobacillus bactericidin is not identical with salivary amylase or lysozyme.

The author wishes to thank Dr. Russell S. Weiser for his many helpful suggestions and valuable criticism in the preparation of this text. The technical assistance of Mrs. Kazuko McCormick is gratefully acknowledged.

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THE EFFECTS OF VARIOUS LEVELS OF SWEET PEAS (LATHYRUS ODORATUS) IN THE DIET, WITH AND WITHOUT DIETARY SUPPLEMENTS, ON THE GROWTH OF THE MANDIBULAR RAMUS AND ARTICULATION IN YOUNG RATS

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INTRODUCTION

DESPITE the well-known and striking effects of lathyrism on the skeleton, relatively few reports are available concerning the possible relationship of experimental lathyrism to the oral and maxillofacial regions.

The purpose of the present study was to investigate the effects of various levels of sweet peas, with and without dietary supplementation, on skeletal growth of the mandibular ramus and articulation. Utilizing pair-fed controls and nonsupplemented (P.D. I) lathyrus-fed controls, this study also attempted to evaluate the influence of protein and amino acid supplementation on the changes observed.

It has long been known that rats fed on diets containing large amounts of *Lathyrus odoratus* seeds develop bone deformities. These are due to the formation of numerous exostoses, especially at muscle-insertion sites, and to irregular proliferation of cartilage, particularly in the rapidly growing epiphyseal plates.[1, 2, 3] The active principle was first extracted in impure form[4] and, subsequently, it has been possible to demonstrate that β (gamma-L-glutamylamino) propionitrile is present in the seeds of *Lathyrus odoratus* and duplicates the effects of this plant upon the skeleton.[5, 6, 7] In view of the many similarities between experimental lathyrism and specific diseases occurring in man, it appears of practical importance to establish whether the skeleton can be rendered resistant to the actions of a drug such as BAPN.†

Dasler,[8] in 1954, found that in odoratism the percentage of ash in the whole incisors actually showed a significant increase, while the level of ash in the femurs decreased.

The most important oral and cranial changes found in lathyrism are periosteal new bone formation, as voluminous exostoses at sites of muscle insertions to the mandible of rats, the most obvious being at the coronoid, condyloid, and angular processes (Gardner and co-workers,[9] Dasler,[8, 10, 11] and Selye[12, 13]). Other changes are gross bone deformities of the cranium and

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†BAPN is an abbreviation for beta-aminoproprionitrile.

voluminous exostoses of the occipital bone (Gardner and associates[9]), increased ash content of incisors (Dasler[11]), deformation of the mandible of *Amblystoma punctatum* embryos (Levy and Goodman[14]), hyalinization of the periodontal membrane (Carwell,[15] Gardner et al.[9]), degenerative arthritis of the mandibular condyle (Gardner[9]) and proliferation of connective tissue (Gardner[9]).

Selye[12, 13, 21-23] found that with chronic treatment of 2 to 3 months, with small doses of aminoacetonitrile, concurrent administration of somatotropic growth hormone caused excessive growth and enamel defects in the incisor teeth. The incisor teeth showed marked malocclusion with several cross lines of enamel defects and excessive growth in length. The tips of the upper incisors had turned upward and were growing into the palate and lips.

Selye[12, 13, 21-23] also found inhibition of lathyrism by Cortisol. In rats receiving aminoacetonitrile plus Cortisol, the mandible remained essentially normal as compared to the development of voluminous mandibular exostoses in rats receiving aminoacetonitrile alone.

An insight into the diseases influencing the growth of the mandibular ramus and articulation presupposes an understanding of the metabolism of all mesodermal tissues. With this background in mind, the study of the effect of sweet pea feeding, with and without the addition of dietary supplementation, on the ramus and mandibular articulation was undertaken.

EXPERIMENTAL MATERIALS AND METHODS

Ninety-six male albino weanling rats, 45 to 50 grams in weight and of the Holtzmann-Sprague-Dawley strain, were used throughout this investigation. The rats were divided into groups at weaning and placed on various diets containing sweet peas. An additional group of male rats were pair-fed and served as normal littermate controls. Each of the experimental rats was paired with one of the normal controls of the same weight.

Ground Lathyrus Odoratus Diets.—The rats in the various groups were fed diets containing different amounts of *Lathyrus odoratus* (beta-aminopropionitrile), and *Lathyrus odoratus* diets plus dietary supplements (Table I).

TABLE I

PROTECTIVE DIETS

	I	II	III	IV	V	VI	VII
Ground sweet peas	30	30	30	30	30	30	30
Starch	45	15	30	11	36	41	40
Casein	10	40		35	10	10	10
Gelatin			25				
Sucrose	5	5	5	5	5	5	5
Brewer's yeast	4	4	4	4	4	4	4
Salt mixture	4	4	4	4	4	4	4
Cod liver oil	2	2	2	2	2	2	2
Glutamine				5	5		5
Cysteine HCl				4	4	4	

The feedings were continued for different periods, utilizing the various experimental and control diets which are described in detail later. For control (edible pea) and experimental sweat pea diets (S.P. I, II, III), the feedings were continued for 1, 2, and 3 weeks, respectively. For diets P.D. I to P.D. VII, the feedings were continued for 56 days. The skulls were removed, and fixed in Zenker-formalin solution for one day, or in Bouin's solution for 2 days.

The experimental diet was as follows:

1. *Lathyrus odoratus* (sweet pea) meal, 50 per cent; casein,* 10 per cent; cornstarch, 25 per cent; sucrose, 5 per cent; brewer's yeast powder (dried), 4 per cent; salt mixture (Hubbell[16]†), 4 per cent; cod liver oil, 2 per cent.

The control diets were as follows:

1. This control diet contains the same ingredients as in diet I of the experimental diets but replaces the *Lathyrus odoratus* meal by a 50 per cent dried and ground commercial edible pea meal.

2. Milled Rockland rat diet. In this section of the present study, mixtures of dietary ingredients were fed in excess together with *Lathyrus odoratus* meal to determine whether they would enhance or suppress the development of lathyrism.

The basal diet (P.D. I)‡ contained 30 per cent sweet pea meal and 45 per cent cornstarch. Additional supplements were incorporated by replacing an equivalent amount of starch. All diets contained casein, gelatin, glutamine, or cysteine.

The protective diets (Lathyrus odoratus 30 per cent, plus dietary supplements) were as follows:

P.D. I. This is a control diet for all protective groups. It consists of ground sweet peas, 30 per cent; casein, 10 per cent; starch, 45 per cent; sucrose 5 per cent; brewer's yeast, 4 per cent; salt mixture (Hubbell[16]), 4 per cent; and cod liver oil, 2 per cent.

The experimental diets with increased supplementation are as follows:

P.D. II. Ground sweet peas, 30 per cent; casein, 40 per cent; starch, 15 per cent; sucrose, 5 per cent; brewer's yeast, 4 per cent; salt mixture (Hubbell[16]), 4 per cent; and cod liver oil, 2 per cent.

P.D. III. Ground sweet peas, 30 per cent; gelatin, 25 per cent; sucrose, 5 per cent; starch, 30 per cent; brewer's yeast, 4 per cent; salt mixture (Hubbell[16]), 4 per cent; and cod liver oil, 2 per cent.

P.D. IV. Ground sweet peas, 30 per cent; casein, 35 per cent; glutamine, 5 per cent; cysteine HCl, 4 per cent; sucrose, 5 per cent; starch, 11 per cent; brewer's yeast, 4 per cent; salt mixture (Hubbell[16]), 4 per cent; and cod liver oil, 2 per cent.

*The casein was plain, untreated casein (not vitamin-free).
†The salt mixture was that devised by Hubbell, Mendel, and Wakeman (1937).
‡P.D. is used as an abbreviation for "protective diet."

P.D. V. Ground sweet peas, 30 per cent; casein, 10 per cent; glutamine, 5 per cent; sucrose, 5 per cent; cysteine HCl, 4 per cent; starch, 36 per cent; brewer's yeast, 4 per cent; salt mixture (Hubbell[16]), 4 per cent; and cod liver oil, 2 per cent.

P.D. VI. Ground sweet peas, 30 per cent; casein, 10 per cent; sucrose, 5 per cent; cysteine HCl, 4 per cent; starch, 41 per cent; brewer's yeast, 4 per cent; salt mixture (Hubbell[16]), 4 per cent; and cod liver oil, 2 per cent.

P.D. VII. Ground sweet peas, 30 per cent; casein, 10 per cent; glutamine, 5 per cent; starch, 40 per cent; sucrose, 5 per cent; brewer's yeast, 4 per cent; salt mixture (Hubbell[16]), 4 per cent; and cod liver oil, 2 per cent.

After sacrifice and fixation, the upper and lower jaws were dissected free from the skulls, so that tissue sections consisted of the mandibular ramus and articulation. These were embedded separately in individual blocks.

All histologic sections were cut consecutively at 6 to 8 μ and stained either with hematoxylin and eosin, Mallory's phosphotungstic acid hematoxylin, van Gieson's stain, periodic acid-Schiff stain, or impregnated with silver according to the method of Gomori. After mounting in Canada balsam, the cover slips were weighted down to obtain flat sections.

RESULTS

Mandibular Ramus and Articulation (Protective Diets).—

By means of a macroscopic examination of the mandibular ramus and articulation, it was readily apparent that the mandibles of the nonsupplemented animals in protective-diet group I were more severely deformed than those of the supplemented rats in groups P.D. II to P.D. VII (Table II).

In the animals fed P.D. I, exostoses developed at muscle-insertion sites, as diffuse periosteal new bone formations which were all more intense than in the rats which received dietary supplementation during the 8 weeks of the experiment. The periosteum of the ramus showed prominent fibroblastic proliferation with excessive widening along the medial surface of the ramus (Fig. 1). The major feature was proliferating fibroblastic connective tissue (Fig. 3). This tissue in itself was nonspecific; however, most areas contained zones with irregular trabeculae of osteoid or bone.

These areas showed well-formed and moderately thick anastomosing bone trabeculae containing "lines of force" (Fig. 5). The lesions occurred in the periosteum of the ramus, and the osseous component was in the form of small masses which were surrounded by the proliferating spindle cells. Mitotic figures were found in the actively proliferating lesions of the periosteum. The fibro-ostosis which occurred in this instance produced masses of osteoid tissue with proliferating fibroblastic tissue between the masses of bone. This pattern was seen in lesions along the ramus of the mandible.

During the early stages of vascular impairment there was capillary anoxia with leakage of plasma which hydrated the stromal matrix adjacent

TABLE II

COMPARISON OF CHANGES IN THE MANDIBULAR RAMUS AND MANDIBULAR ARTICULATION OF MALE RATS FED PROTECTIVE LATHYRISMIC DIETS FOR 8 WEEKS

PROTECTIVE GROUP	P.D. I	P.D. II	P.D. III	P.D. IV	P.D. V	P.D. VI	P.D. VII
Fibroblastic proliferation and thickened periosteum along medial surface of ramus	prominent	absent	absent	absent	absent	absent	absent
Subperiosteal new bone formation along medial surface of the ramus below neck of condyle (exostoses)	present	absent	absent	absent	absent	absent	absent
Subperiosteal new bone formation at ventral surface of angular process (exostoses)	present	absent	absent	absent	absent	absent	absent
Lateral edge of roof of mandibular articulation	exuberant exostoses	absent	absent	absent	absent	absent	absent
Ectopic bone formation in temporalis muscle adjacent to mandibular roof	present	absent	absent	absent	absent	absent	absent
Presence of basophilic bone globules in newly formed lathyrismic bone	prominent	absent	absent	absent	absent	absent	absent
Osteoporosis of ramus with dilatation of blood vessels in solid bone	prominent	absent	absent	absent	absent	absent	absent

to capillaries. This hydration stimulated the fibrous stroma to proliferate and lay down excessive collagen bundles perivascularly in the proliferating periosteum (Fig. 2).

Histologic examination revealed changes in the ramus consisting of dust-like, small to large basophilic granules or globules within the widened proliferating periosteum and in the newly formed periosteal bone or exostosis. They developed initially in the fibrous periosteal tissue not yet ossified. There were innumerable globular formations that took the basophilic stain, wherever new bone formations occurred. It is felt that the development of this peculiar type of bone structure was quite characteristic only for animals with severe lathyrism since it never occurred in the littermate controls. The exostoses and periosteal new bone formation developed as an exuberant bone formation only at the sites of attachment of the masseter and internal pterygoid muscle to the angular process and of the external pterygoid muscle to the medial surface of the ramus of the mandible.

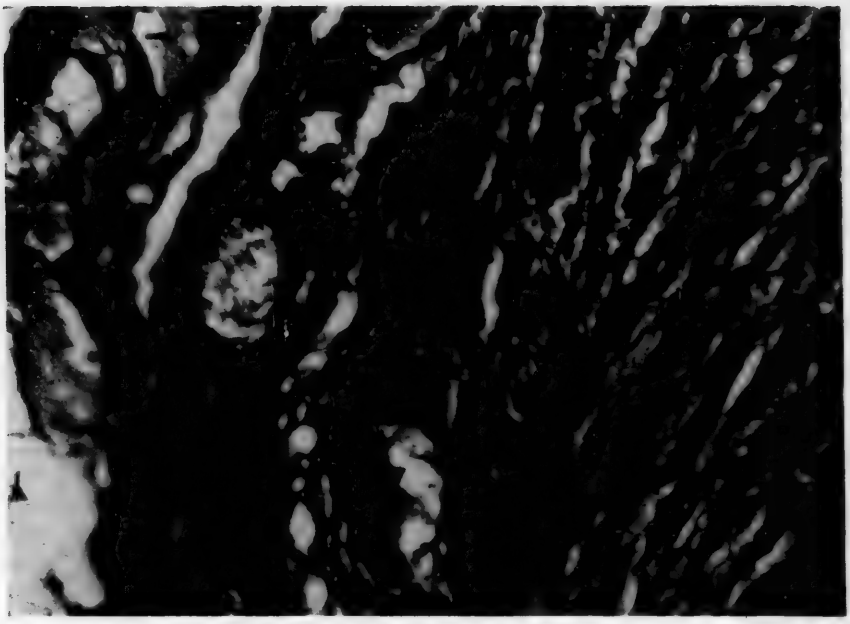

Fig. 1.—Photomicrograph of a mediolateral section of the periosteum along the medial surface of the ramus of the mandible in a male rat fed a 30 per cent sweet pea diet (P.D. I) for 8 weeks. There is an increase in fibroblastic activity during which abundant collagen is laid down. The periosteum is greatly increased by fibrous thickening. The number of collagen fibers per unit area is increased. Small foci of an eosinophilic material containing few disoriented fibrils are present in this periosteal connective tissue. (Masson's stain; orig. mag. × 600.)

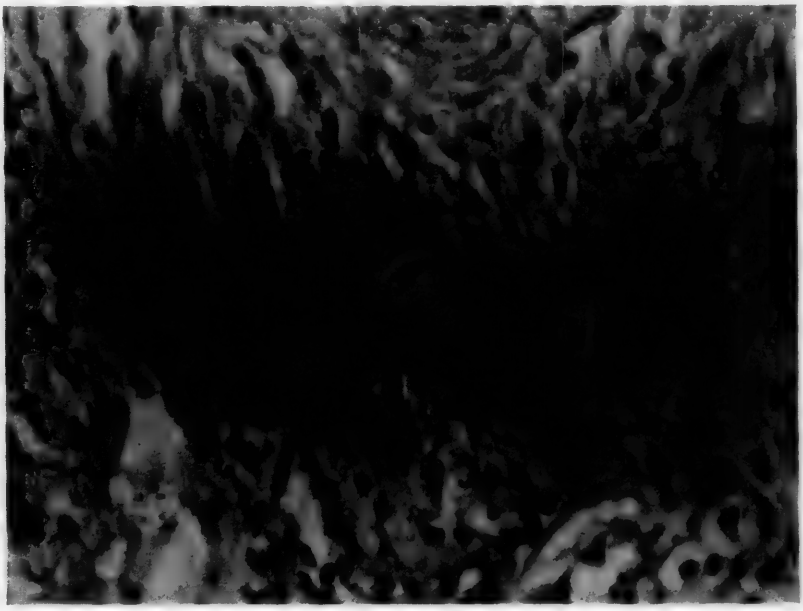

Fig. 2.—Photomicrograph of a mediolateral section of the periosteum along the medial surface of the ramus of the mandible in a male rat fed a 30 per cent sweet pea diet (P.D. I) for 8 weeks. Note the increased deposition of collagen in this proliferating periosteum. The number of collagen fibers and fibroblasts per unit area is increased. Elongated nuclei of fibroblasts are numerous in an eosinophilic matrix. (van Gieson's stain; orig. mag. × 900.)

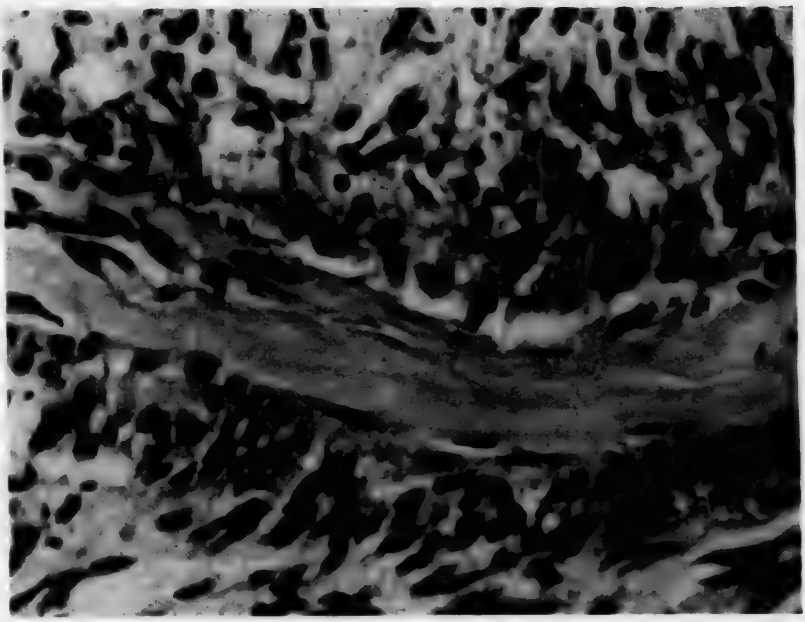

Fig. 3.—Photomicrograph of a mediolateral section of the periosteum along the medial surface of ramus of the mandible of a male rat fed a 50 per cent sweet pea diet (S.P. III) for 3 weeks. Note the proliferation of connective tissue which is composed of rather immature, tightly packed, spindle cells. The fibroblasts show a tendency to lay down broad areas of an eosinophilic collagenous tissue. The primary component of this lesion is an abundant connective tissue frequently arranged in interlacing bundles. (Hematoxylin and eosin stain; orig. mag. × 900.)

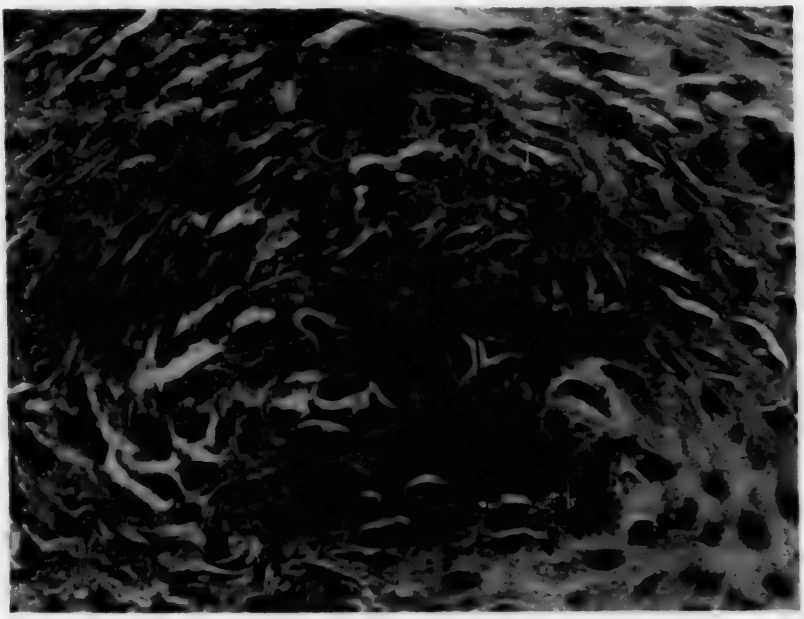

Fig. 4.—Photomicrograph of a mediolateral section of the periosteum along the medial surface of the ramus of the mandible of a male rat fed a 30 per cent sweet pea diet (P.D. I) for 8 weeks. Note that the connective tissue cells round up, the nuclei enlarges, and the intercellular fibrils thicken and stain deeply with eosin. The intercellular substance is increased in amount and is calcified. (Hematoxylin and eosin stain; orig. mag. × 500.)

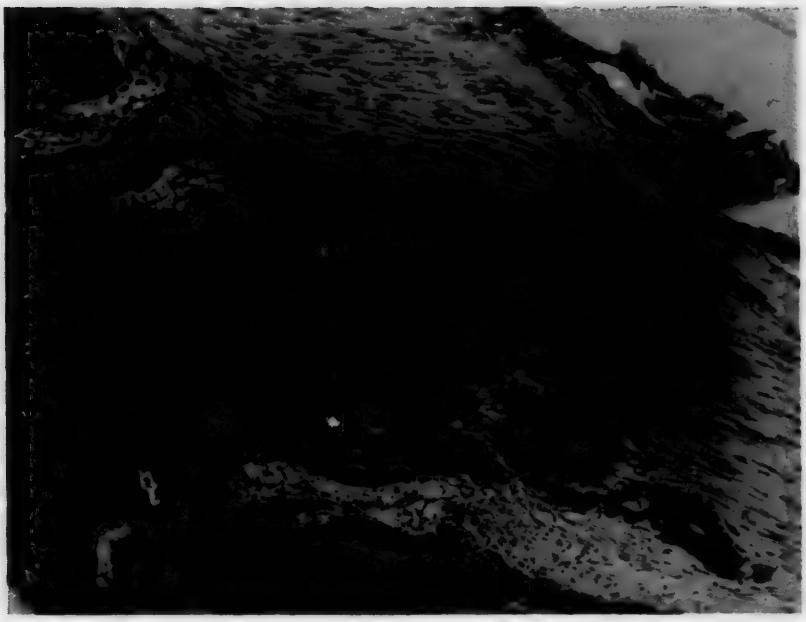

Fig. 5.—Photomicrograph of a mediolateral section of the periosteum and ramus on the mandible of a male rat fed a 30 per cent sweet pea diet (P.D. I) for 8 weeks. Note the fibro-ostosis, a direct metaplasia from connective tissue to bone within the proliferating periosteum. The incarcerated connective tissue cells are indistinguishable from osteocytes. The periosteum appears as an eosinophilic homogeneous connective tissue. This lathyrismic new bone formation is oriented along the lines (direction) of muscle pull at this site of muscle insertion to the mandible. (Hematoxylin and eosin stain; orig. mag. × 80.)

Fig. 6.—Photomicrograph of a mediolateral section of the fibro-ostosis (Fig. 5) along the ramus of the mandible of a male rat fed a 30 per cent sweet pea diet (P.D. I) for 8 weeks. Note the deposition of numerous basophilic bone globules of lathyrism in the intercellular matrix which shows an uneven basophilia, indicative of calcification. These may be lathyrismic corpuscles of incipient calcification. The incarcerated connective tissue cells become osteocytes. (Hematoxylin and eosin stain; orig. mag. × 750.)

At the angular process, the subperiosteal new bone formation was of the osteophytic type. It was surrounded by a widened and hyperemic periosteum. This osteophytic growth of bone had enlarged and greatly deformed the angle of the mandible. The remaining surfaces of the ramus which were free of muscle attachments were also entirely free of subperiosteal new bone formation. The newly formed bone contained trabeculae that were lined up along the direction of muscle pull and appeared to invade the surrounding muscles, more or less diffusely, so that it was difficult to dissect the skeletal structures from the adjacent soft tissues. The peculiar lathyrismic bone change was particularly manifest in the mandibles, presumably because they are the site of many muscle insertions and thus have a great predisposition to exhibit signs of bone lathyrism. In sections stained with hematoxylin and eosin, the

Fig. 7.—Photomicrograph of a mediolateral section of a fibro-ostosis or exostosis along the ramus of the mandible of a male rat fed a 30 per cent sweet pea diet (P.D. I) for 8 weeks. Note the basophilic bone globules of lathyrism in the mature bone. They form a complete circle about a small central nidus. The periphery of the globule and the nidus stain more intensely than the remaining portion. The intercellular substance is calcified. (Hematoxylin and eosin stain; orig. mag. × 750.)

granules and globules in the newly formed bone stained blue and their outlines varied from round to somewhat sharply delineated. The size of the granules varied from dust particle size to 1 or 2 μ in diameter (Fig. 6). In the larger globules which were easily distinguishable, the center was comparatively light, as if it were, so to speak, hollow (Fig. 7). Within the exostoses, several globules appeared to coalesce into a berry-like structure. This may have been due to the great abundance of globules and the closer approximation in some areas of the newly formed bone. Frequently they appeared

to be arranged in irregular chains which seemed to follow the course of the bone canaliculi into which the osteocytes extended their processes. The globules also exhibited a peculiarity in that they appeared to aggregate around the lacunae of osteocytes (Fig. 4). In an examination of serial sections of normal littermate rats and of other normal rats at varying ages, none of these sections showed any comparable development or formations of basophilic granules or globules. In the mandibular articulation, new bone formation was also particularly evident with an intense and irregular proliferation of new bone along the lateral edge and margin of the roof of the articulation. This is the site of attachment of the temporalis muscle. These changes were incomparably more pronounced in P.D. I animals than in any protective group fed dietary proteins in addition to sweet peas.

The irregular and excessive growth of bone at the lateral edge of the articular roof thickened as it advanced inferiorly to cover the upper third of the ramus of the mandible. Immediately superior to the lateral surface of the articular roof, ectopic bone formation developed within the temporalis muscle. It developed within the muscle close to the squamous bone but not in continuity with it.

The ramus of this group showed a loss of normal modeling activities with only endosteal resorption in evidence. There was a definite osteoporosis of the ramus of the mandible, composed of abnormal periosteal ramal bone formation, reduced number of trabeculae persisting, endosteal resorptive activities, and an increase in the hematopoietic bone marrow. The remaining cells lining the trabecular surfaces were small and flattened, indicating that no endosteal bone formation was taking place at the time of sacrifice. The osteoporosis became accentuated with continued feeding of the nonsupplemented lathyrus diet which at the termination of the experiment led to severe endosteal breakdown of bone tissue.

In the animals fed P.D. II, the periosteum was of average width along the entire ramus of the mandible. There was no evidence of abnormal fibroblastic proliferation within the periosteum. Subperiosteal new bone formation was entirely lacking along the medial surface of the ramus below the neck of the condyle of all animals of this group. The following experimental findings, which were so predominant in the animals of protective diet I group, were absent in the animals of this group: (1) subperiosteal new bone formation at the ventral surface of the angular process of the mandible; (2) exuberant exostoses at the lateral edge of the mandibular roof; (3) ectopic bone formation in the temporalis muscle adjacent to the mandibular roof; (4) presence of basophilic bone grains and globules in newly formed lathyrismic bone; and (5) osteoporosis of the ramus.

In the groups fed protective diets III, IV, V, VI, and VII, none of the experimental findings so prominent in P.D. I were visible. From this and other observations, it is evident that dietary supplementation of lathyrismic diets markedly inhibited the development of bone lathyrism when the supplements were gelatin, casein, glutamine, and cysteine.

Mandibular Ramus and Articulation (Sweet Pea Diets).—

Upon histologic examination, it was found that the severity of changes throughout the ramus, that is, the proliferation of the connective tissue of the periosteum and its transformation into bone, was much more intense when the feeding period was prolonged (Table III).

TABLE III
COMPARISON OF CHANGES IN THE MANDIBULAR RAMUS AND MANDIBULAR ARTICULATION OF MALE RATS FED A 50 PER CENT SWEET PEA DIET (EXPERIMENTAL) FOR 1, 2, AND 3 WEEKS, RESPECTIVELY

SWEET PEA GROUPS	EXPERIMENTAL DIETS		
	S.P. I	S.P. II	S.P. III
Fibroblastic proliferation and thickened periosteum along medial surface of ramus	absent	moderate	prominent
Subperiosteal new bone formation along medial surface of ramus below neck of condyle (exostoses)	absent	present	prominent
Subperiosteal new bone formation at ventral surface of angular process (exostoses)	absent	present	prominent
Lateral edge of roof of mandibular articulation	absent	small exostoses	exuberant exostoses
Ectopic bone formation in temporalis muscle adjacent to mandibular roof	present	moderate	advanced
Presence of basophilic bone globules in newly formed lathyrismic bone	absent	present	prominent
Osteoporosis of ramus with dilatation of the blood vessel channels in solid bone	absent	present	prominent

In the ramus of rats fed the nonsupplemented sweet pea diet (S.P. I) for one week, the periosteum appeared quite normal. The total width was not increased and no development of subperiosteal new bone formation had occurred. Furthermore, the angular process was free of exostoses and the roof of the mandibular articulation was of normal morphology. The trabeculae of bone were subjected to a careful study for the basophilic granules described in animals fed P.D. I. Observations indicate that they were not present in this group of rats, either as dust-like structures or as larger basophilic granules. Osteoporosis was absent from the ramus of all animals in this group. Ectopic bone formation was not evident in the temporalis muscle superior to the mandibular roof. The lateral edge of the roof of the mandibular articulation was not thickened by an overgrowth of bone.

In the ramus and mandibular articulation of rats fed sweet pea diet II, fibroblastic proliferation became apparent. Mitosis was evident and a young connective tissue was developing, showing very few collagenous fibers. This fibroblastic proliferation occurred at sites of tendon or muscle insertions to the mandible and produced a widening of the periosteum in these locations. Subperiosteal new bone formation started to develop in the animals of this group upon the medial surface of the ramus immediately below the neck of

the condyle. The trabeculae that formed in this widened and proliferating periosteum were oriented principally along the lines of tension exerted by the muscle attachments of the external pterygoid.

Subperiosteal new bone formation was also apparent, although only moderate in amount at the ventral surface of the angular process. This exostosis produced an irregular and very deformed angular process, presenting the appearance of osteophytic bone. From the lateral edge of the roof of the mandibular articulation, a moderate exostosis had developed at the attachment of the temporalis muscle. This new growth of bone extended inferiorly and covered the upper fourth of the condylar process, enlarging the area of the mandibular roof.

Ectopic bone formation began to develop in the temporalis muscle immediately superior to the roof of the mandibular articulation. It began as a young, proliferating connective tissue, replacing and invading the temporalis muscle at some distance from the bony roof of the mandibular articulation. In selected areas of this connective tissue, within the substance of the temporalis muscle, trabeculae of intramembranous bone were forming. The marrow spaces were occupied by a loose proliferating connective tissue. In the subperiosteal new bone formation, small sand-like grains of basophilic material were evident in the bone matrix. This appeared to be a characteristic only of new bone formed in the presence of the lathyrus toxin. Osteoporosis was moderate in the ramus of all animals of this group. Osteoclasts were active endosteally with a reduction in the size and number of bone trabeculae. The bone marrow was increased in amount and was of the hematopoietic type.

In the ramus of animals given the 50 per cent sweet pea diet for a period of 3 weeks, very pronounced changes were evident in the ramus and mandibular articulation. At the sites of tendon and muscle insertions to the mandible and articulation, the periosteum became greatly widened due to the proliferation of fibroblasts stimulated by the sweet pea toxin accompanied by mechanical or topical stress. Histologic examination also showed a definite difference between the structure of the ramus in this experimental group and its littermate controls. The proliferation of fibrous and osseous tissue was excessive in this group when compared with the controls. Periosteal new bone had formed at the sites of tendon and muscle insertions. The characteristic basophilic globules of experimental lathyrism were obvious by the third week of experimental feeding. These varied in size from dust-like, just visible, roundish particles or granules to bodies or structures having a diameter of about 1 to 2 μ, and they occurred most frequently at areas where the proliferating periosteum was undergoing direct transformation into membranous bone. The bone globules stained intensely with hematoxylin, or after being mordanted with 0.8 per cent periodic acid-Schiff stain for 5 minutes. The cortical bone of the ramus consisted of circumferential lamellae, which ran parallel to the periosteum, while haversian canal systems and

resting lines were very poorly developed. Hence, it was particularly striking that in all rats of the group fed S.P. III, an extraordinarily polymorphic mottling of lamellar systems had arisen. There was Paget-like bone consisting of dense bone with very thick bone trabeculae containing mosaic patterns in the upper and lower portions of the ramus. The resting lines between these bone trabeculae were wide and stained deep blue with hematoxylin. The mosaic patterns were primarily present at areas of major stress upon the ramus. Such areas were present at the insertions of the external pterygoid and internal pterygoid muscles on the medial surface of the ramus, and where the masseter muscle inserts into the angular process of the mandible. However, bone resembling that seen in Paget's disease was visible, extending throughout the width and length of the ramus in several animals of this group. The vascular canals that carry the blood vessels were greatly dilated. The osteocytes were quite irregularly arranged. The ramus was eroded by deep Howship's lacunae on the periosteal and endosteal surfaces. The bone marrow remained essentially normal, although increased in quantity. The prominent areas of subperiosteal new bone formation upon the ramus and mandibular articulation were located at the medial surface of the ramus below the neck of the condyle, at the ventral surface of the angular process, and at the lateral border of the mandibular roof. The spaces between the cancellous bone trabeculae were filled with partly fibrous, partly myeloid bone marrow.

Ectopic bone formation was well advanced within the temporalis muscle adjacent to the mandibular roof. These ectopic structures were well organized with regular intramembranous osteogenesis. The osteogenesis induced in this portion of the temporalis muscle was extremely voluminous after the third week of experimental feeding. The intercellular substance was filled with these basophilic granules and globules which appear to be specific for experimental lathyrism. Osteoporosis was well advanced in the ramus by the end of the third week of the nonsupplemented sweet pea feeding.

The ramus of this group had thinner trabeculae and large medullary spaces. The number and size of the osteoclasts were increased. In some cases, a thin transverse lamina of bone was deposited on part of the lateral inferior border of the condylar cartilage. The bony trabeculae were fewer and very irregular in their orientation when compared to the controls.

DISCUSSION

Under the conditions of this investigation, sweet peas alone produced skeletal alterations with no obvious derangements in the structure of the bone marrow. On the other hand, combined treatment with sweet peas and dietary supplementation, consisting of proteins and amino acids, resulted in very minimal skeletal deformities with normal hematopoietic bone marrow.

In connection with the problem of aging of mesenchymal tissues, it is of interest that many of the lesions induced by nonsupplemented sweet pea feeding resemble those seen in senescent rats. Among these alterations are

(1) osteoporosis of the ramus with dilatation of the blood vessel channels in solid bone, (2) thickened and proliferating periosteum, and (3) periosteal new bone formation at sites of tendon and muscle insertions.

It is also noteworthy that the height of the ramus in the nonsupplemented sweet pea groups, S.P. II, S.P. III, and P.D. I, was obviously shorter than those of the supplemented lathyrus-fed groups. Thus, the sweet pea inhibited both anabolism and growth in height of the ramus, under this particular experimental condition of lathyrism.

It is, of course, quite impossible to definitely verify whether the alterations due to nonsupplemented sweet pea feeding in this investigation actually correspond to premature aging. Further work will be necessary to show whether toxic food products or toxic endogenous by-products of metabolism can result in the development of changes usually ascribed to senility.

It was deemed advisable, in view of the current reports in the literature that a disturbance in collagen metabolism or connective tissue metabolism is involved in lathyrism, to feed proteins with hydroxyproline content to rats receiving sweet pea diets. Gelatin was selected as a good source of hydroxyproline. The protein and amino acid supplements were utilized to protect the rat against experimental lathyrism because collagen differs so markedly from other natural proteins in its hydroxyproline content. This study investigated the role of proteins and other supplements, such as amino acids, as possible etiologic factors.

Casein prevented the formation of lesions of the ramus and mandibular articulation, even in the most advanced cases. Palpable exostoses of the jaws were absent, the jaws remaining indistinguishable from those of normal rats.

Gelatin also prevented the formation of lesions of the ramus and mandibular articulation. Some minor irregularity, however, existed histologically in the ramus and mandibular articulation at the sites of muscle attachments.

The apparent superiority of the protective action of casein over that of gelatin, however, is probably not a result of the better growth on the high casein diet, since other experiments indicate that the more rapidly growing animal develops symptoms of lathyrism at least as fast as the slower growing animal. The increased rate of growth of the rats receiving the high casein diet, as contrasted to those receiving the high gelatin diet, is no doubt due to the amino acid deficiencies of gelatin. It seems impossible that a deficiency of an essential amino acid could be involved in lathyrism because the animals continue to grow until a terminal stage is approached. Furthermore, the symptoms of lathyrism are profoundly different from those produced by any known amino acid deficiency.

In this experiment, proteins (casein, gelatin) and amino acids (L-glutamine and L-cysteine) delayed the onset of lathyrism, but allowed the production of a mild form of the disease. Glutamine appears to be involved in the metabolic defect, since glutamine has been reported to act as an accelerator of chondroitin sulfate synthesis and probably plays a part in the biosynthesis of mammalian mucopolysaccharide (Bostrom, Roden, and Vestermark[17, 18]).

It has been pointed out by many investigators that lathyrism due to the feeding of sweet peas to rats appears specifically to influence mesodermal tissues. The metabolic defect has been postulated to be localized in the ground substance, which is rich in mucopolysaccharide.[19, 20]

When 5 per cent L-glutamine was incorporated into the 30 per cent sweet pea diet, it caused a delay in the onset and severity of changes in the ramus and mandibular articulation. The rats receiving glutamine were not only larger than the nonsupplemented P.D. I animals, but their fur was sleek, they had better muscle tone, and appeared to be more active in their cages.

When fresh diets, containing both cysteine and glutamine, were fed to rats, greater protection was present against the lesions of the ramus and mandibular articulation than occurred with either supplement alone.

The diet containing 5 per cent glutamine plus 30 per cent casein appeared to give somewhat better protection than the diet containing 30 per cent casein without the addition of glutamine. If one increases the glutamine level to 10 per cent, no apparent additional protection occurs than is present with a level of 5 per cent. The beneficial effect of glutamine in these diets is not very readily explained. If one adds glutamine to a diet* without added sweet peas, no growth stimulating effect is apparent. However, when the glutamine is added to a *Lathyrus odoratus* diet, a growth stimulating effect is present. The protective action of glutamine may be related to its possible role in chondroitin sulfate and mucopolysaccharide synthesis. The nature of this investigation has not permitted any definitive answer for this question. Further research is necessary in order to provide an insight into this question.

The addition of 5 per cent L-cysteine-HCl plus 5 per cent L-glutamine to a 30 per cent sweet pea diet had definite growth stimulating effects; however, protection against skeletal changes in the mandibular ramus and articulation were greater than with either of these supplements alone.

A complete understanding of the pathogenesis of the changes in the total skeleton, as manifested through the mandibular ramus and articulation, will facilitate greatly the determination of the basic cause of this condition, as well as determine what phase of development of bone tissue is adversely influenced.

It is evident by the findings in this investigation that beta-aminopropionitrile in the sweat pea induces some metabolic disturbance, which predisposes the rat to the formation of subperiosteal new bone at the sites of muscle and tendon insertions. In and around these areas of attachment, the periosteum showed excessive fibroblastic proliferative changes with bone proliferation to form exostoses. In general, the fibroblastic and bone proliferation were absent in the groups of rats fed the supplemented protein diets. The newly formed osseous tissue possessed a comparatively hard consistency and true ossification was evident.

The mechanism of this overproduction of bone is not absolutely clear. Initially, however, beta-aminopropionitrile produces some systemic metabolic change, which predisposes bone at sites of stress to excessive reaction to tension, forces, or injury.

*Rockland rat.

Under the influence of beta-aminopropionitrile, the excessive new formation of bone appears to develop only where there is some special mechanical stress, for example, at muscle and tendon insertions. Presumably, the mechanical pull upon the bone stimulates osteogenesis through the production of topical stress. Therefore, the combination of local stress and sensitization by beta-aminopropionitrile in sweet peas is presumably of pathogenic importance. The presence of the great number of basophilic bone globules is the only anomaly, in the intercellular substance of the new formed bone, characteristic of beta-aminopropionitrile intoxication. But it still remains to be seen whether these globules, grains, or bodies play a role in the production, or are merely the consequence of lathyritic bone lesions.

The findings in the ramus of nonsupplemented lathyrus-fed animals confirmed a development of excessive, calcified bone tissue at muscle insertion sites and underneath the periosteum of the ramus and mandibular articulation. All of the bone changes were virtually absent in the ramus of rats treated with dietary supplements although, in some animals, occasional minor irregularities in bone structure could be demonstrated upon careful histologic investigation. It is also noteworthy that topical stress in bones plays an extremely important part in the development of skeletal lathyrism. It had long been known that, in lathyritic rats, exostoses are most common at tendon-insertion sites.

Throughout this investigation, in the ramus and mandibular articulation of lathyritic rats, a nonsupplemented sweet pea diet produced exostoses at all tendon-insertion sites. This confirms the findings in the long bones of previous investigators.

The sweet pea caused other severe derangements in the architecture of the ramus and mandibular articulation. There was a tendency to irregular arrangement of osteocytes and some increase in the thickness of compact bone at tendon-insertion sites. This led to a weakening of ramal structure, due also to osteoporosis caused by the enlargement of vascular channels.

SUMMARY

Experiments on rats indicated that systemic treatment with beta-aminopropionitrile from sweet peas produces a metabolic defect which causes the mandible and skeleton to respond with new bone formation in regions of mechanical topical stress.

Experiments on rats showed that moderate doses of beta-aminopropionitrile in sweet peas, which in themselves elicited lesions characteristic of skeletal lathyrism, produced very minor lesions with no morbid changes when fed in combination with dietary supplementation of proteins and other substances (amino acids).

Most important among these alterations were: thickening and proliferation of the periosteum, subperiosteal new bone formation at muscle insertion sites on the ramus and angular process of the mandible and on the roof of the mandibular articulation, ectopic bone formation in temporalis

muscle, presence of basophilic bone globules (positive to periodic acid-Schiff stain) in newly formed lathyrismic bone, and osteoporosis with dilatation of the blood vessel channels in solid bone.

Certain basophilic granules and globules were described in the intercellular substance of newly formed bone of mandibles of rats fed nonsupplemented sweet pea diets. These globules appeared to be very specific for experimental lathyrism.

The osteoporosis of the ramus became accentuated with continued protein deprivation and led to severe breakdown of bone tissue in the nonsupplemented groups where one may assume an increased rate of resorption took place.

Dietary supplementation with proteins and amino acids greatly diminished the development of bone lathyrism that is normally produced by aminopropionitrile *(Lathyrus odoratus)* in the rat. The amino acids, L-cysteine-HCl and L-glutamine, gave protection against onset and severity of skeletal lesions in the ramus and mandibular articulation in rats fed sweet peas.

Glutamine had a growth stimulating effect, in addition to delaying the onset and severity of changes in the ramus and mandibular articulation.

The protein supplements, casein and gelatin in high levels, delayed the onset and reduced the severity of lesions of the ramus and mandibular articulation. Casein exerted a greater protective action than gelatin. The protein and amino acid supplements restored the ability of the lathyrismic rat to diminish osteoporosis and osteoclastic activity, to stimulate normal apposition of bone, and to prevent new bone formation at sites of muscle insertions.

The lack of development of exostoses, therefore, may be used as an early and reliable indicator of the success of supplemental feedings of proteins and amino acids to lathyrismic diets.

The finding of a protective agent was deemed of considerable biologic importance since a possible relationship exists between experimental lathyrism and a number of human diseases of unknown etiology.

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THE EFFECT OF OAT HULLS ON THE DENTAL CARIES EXPERIENCE IN RATS

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IT has been suggested that oat hulls are anticariogenic, since their addition to the diet of laboratory animals has been observed to reduce both the incidence and severity of the carious lesions.[1] The actual identity of this factor or factors present in the oat hulls which confer this anticariogenicity to the diet is not known, although certain evidence suggests that it is a nitrogenous base conjugated with palmitic acid.[2] Additional studies in this interesting nutritional area are needed in order to determine the effect that other dietary vehicles have upon the relationship between oat hulls and dental caries. Also, in the present study, the effect of adding oat hulls to the diet was investigated at two different dietary levels since previous experience had shown that certain substances may increase the caries experience, or have less of an anticariogenic effect, instead of reducing the caries rate, when the dietary content is increased.[3]

EXPERIMENTAL

A total of 90 weanling rats of the Sprague-Dawley strain was divided into three groups in which the sexes of the animals were balanced as closely as possible. Group I received a standard cariogenic diet composed of: (in per cent) yellow corn grits, 52.7; ground yellow whole corn, 11.3; powdered whole milk, 30.0; alfalfa, 4.8; iodized sodium chloride, 1.0; and irradiated yeast, 0.2. Groups II and III received the same diet with 5 per cent or 10 per cent substitution of oat hulls. The dietary component which was replaced was in both cases whole milk powder and corn grits, 50:50. After 120 days on the ad libitum regimens and fluoride-free water, the rats were sacrificed. The animals were housed, in pairs, in raised screen cages in an air-conditioned room. The heads were examined for dental caries by methods previously described.[4]

DATA AND DISCUSSION

The data which were obtained in this study are found in Table I. Neither of the two diets which contained oat hulls had any significant effect

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on the body weight gain of the animals. All of the animals gained weight normally in comparison to the control group for which much previous experience is available concerning growth and reproduction.

TABLE I

GROUP	ANALYSIS OF DIET P (GM. PER CENT)	CA (GM. PER CENT)	F (PPM)	SEX	NO. OF ANIMALS FINISHING STUDY	WT. GAIN (GM.)	CARIOUS RATS (PER CENT)	MOLARS AFFECTED	MEAN NO. CARIOUS LESIONS*	SEVERITY‡	REDUCTION IN CARIES§ (PER CENT)	p
(I) Control	0.30	0.30	1.00	♂	12	271	93	4.1	8.1 ± 0.4†	2.64	—	—
				♀	18	152						
(II) 5% oat hulls	0.26	0.33	0.74	♂	18	261	80	3.5	6.5 ± 0.5	2.02	22	0.01
				♀	16	163						
(III) 10% oat hulls	0.36	0.30	0.22	♂	16	259	68	3.4	5.1 ± 0.5	1.85	39	0.001
				♀	18	155						

*Sexes combined.
†Standard deviation.
‡See J. D. Res. 36: 304, 1957.
§Based upon mean number of lesions.

A significant anticariogenic effect of the oat hulls is seen at both levels used in this study. It is of interest to note that increasing the amount of oat hulls to a level of 10 per cent results in a greater reduction in dental caries than when they were added at the 5 per cent level. Apparently the factor or factors present in the oat hulls is increasingly effective when added in increasing amounts. These results clearly demonstrate the anticariogenic effect of oat hulls, but additional work is needed in order to determine what is present in the oat hulls which confers this anticariogenicity to the diet. Due to the low concentration of fluoride in each of the diets, this element cannot be the contributing factor.

SUMMARY

Oat hulls were substituted at a level of either 5 or 10 per cent in a cariogenic diet, in order to determine their effect on dental caries in the rat. At both levels, the oat hulls significantly reduced the incidence of dental caries. The factor or factors responsible for this anticariogenicity is not known, although it is known not to be fluoride.

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A "CLINICAL RULE": USED AS A MEASURING INDEX IN REPOSITIONING PERIODONTALLY-INVOLVED, MIGRATED TEETH

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INTRODUCTION

PROGRESSIVE, acute, and chronic (mixed) inflammatory disease identified with advanced, long-standing marginal periodontitis, often presents the sequela—migration of individual or segments of teeth with diastemata. When, additionally, there is premature loss of posterior teeth and failure to have them replaced, vertical closure of the maxillomandibular relationship is encouraged, with the lower jaw moving forward and upward to its final brace position associated with the final act of deglutition. Healthy overjet is progressively decreased and increased overbite progressively exaggerated, until the teeth on the anterior segments of the upper and lower jaws are traumatogenically related. Increasing the horizontal stress on these teeth during the functional and contact static positions of the lower jaw (as a result of the foregoing) often acts as a secondary factor in producing or exaggerating diastemata.

With our thinking directed toward the basic prerequisite of healthy and optimal functional capacity for the oral environment, it is recognized that there is need for a clinically reliable index to determine the prognosis for the successful functional repositioning of periodontally migrated teeth. The availability of such an index would fulfill this need.

Successful control of the problems of tooth migration, associated with periodontitis, demands the restoration of healthy anatomic relationships, which, in fact, are the only rational bases upon which optimal functional capacity can be encouraged, disease susceptibility discouraged, and maximum, gainful longevity of the dentition reliably attained. These criteria represent the foundation upon which the clinical "assumption" is predicated.

Clinical experience with the problem of chronic periodontitis, complicated by anterior arch distortion resulting from the pathologic migration of teeth, has suggested the following geometric relationships between (1) the relative measurements of tooth substance and arch substance, and (2) the degree of overbite and the angle of overjet. The latter is determined by relating the clinical crown heights of the contacting lower anterior teeth, from cuspid to

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cuspid (when the jaws are closed in the hinge position) to the inciso- (lower anterior teeth) palato- (upper anterior teeth) distance, measured anteroposteriorly on a horizontal plane.

This paper is concerned with a technic designed in an attempt to formulate these relationships in order to establish useful, expedient, duplicatable clinical measurements. The findings are assayed to determine the prognosis for the successful repositioning of periodontally-involved, migrated teeth with diastemata into a healthy arch form with contact point relationship (from upper right cuspid to upper left cuspid tooth) without violating the functional overbite and overjet; the latter are clinically basic criteria for optimal maintenance, without remission.

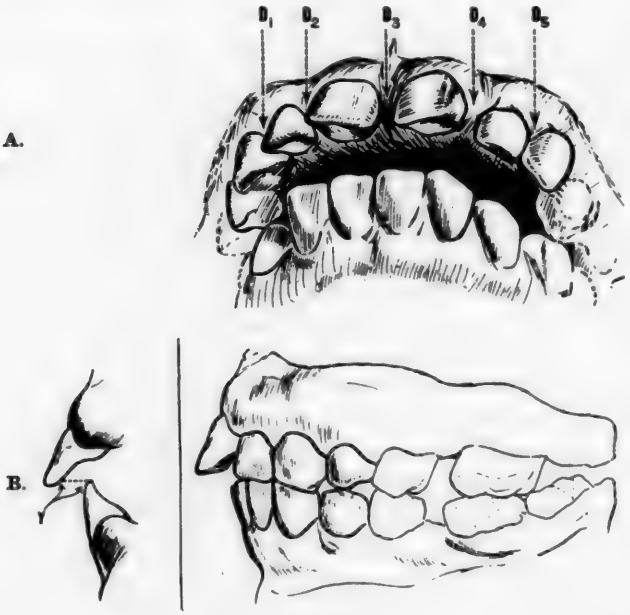

Fig. 1.

FORMULATION

In order to formulate the previously noted geometric relationships one (1) and two (2), account must be taken of the relation of the diastemata D_i ($i = 1, 2, 3, 4, 5$) (Fig. 1, *A*) to "y" (Fig. 1, *B*), which is the distance between the incisal edge level of the two most anteriorly positioned lower teeth and the points on the palatal surfaces of the two most anteriorly positioned upper teeth.

With the initial measurements predicated on the presence of an anatomically integrated, contacting lower anterior segment of teeth, experience in practice provides the bases for the following "Clinical Rule": *If the sum of the distances "D_i" (on the upper jaw) is not greater than the horizontal distance*

"y," when the jaws are closed in the hinge position, it is possible functionally to re-position the upper anterior teeth, eliminating the existing diastemata, and establish healthy interproximal contact-point relationship. The condition under which the above rule is assumed to hold is indicated in Fig. 1, *A* and depends on the relationship of the incisal edge level of the lower anterior teeth to the distance "y."

A tangent to the arc formed by the incisal edges of the lower, most anterior-positioned teeth and perpendicular to "y" must be behind a cord joining the cuspid teeth 1 and 6 (Fig. 2), when the lower anterior teeth are in proximal contact. If this last condition is not fulfilled, the mesiopalatal repositioning of the upper anterior teeth cannot be effected to optimum level.

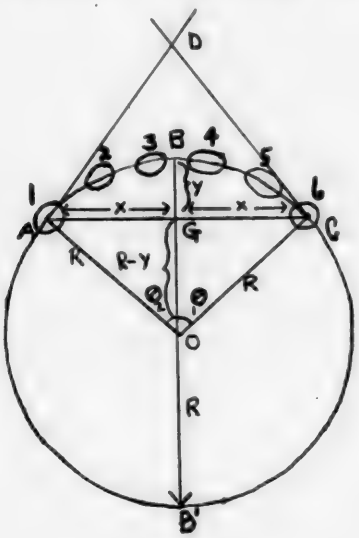

Fig. 2.

These circumstances are expressed as follows:

$$\sum_{1=1}^{5} D_1 \leq y \qquad (1)$$

Where the symbol (=) holds when the teeth are repositioned into contact; and the symbol (<) holds when the combined sum measurement of the diastemata at the contact-point levels of the teeth is less than "y."

In order to develop the simple geometric relationships which are consequences of the above "rule," refer to Fig. 2.

A and C are the positions of the upper cuspid teeth. The incisal edges of the teeth lie along an approximately circular arc $\overset{\frown}{ABC}$. When the teeth have been functionally repositioned, their palatal surfaces will approach line $\overline{AGC}$. The " rule" expressed in terms of Fig. 1 is re-expressed in terms of Fig. 2.

Since

$$\sum_{i=1}^{5} D_i \geq \overset{\frown}{ABC} - \overline{AGC} \tag{2}$$

Eliminating

$$\sum_{i=1}^{5} D_i$$

between Eq. (1) and Eq. (2)

$$\overset{\frown}{ABC} - \overline{AGC} \leq y \tag{3}$$

From Fig. 2

$$\overset{\frown}{ABC} = 2\theta_1 R \tag{4}$$

$$\overline{AGC} = 2X = 2R \sin \theta_1 \tag{5}$$

$$Y = R - R \cos \theta_1 \tag{6}$$

Substituting (4), (5), and (6) into (3):

$$2\theta_1 R - 2R \sin \theta_1 \leq R - R \cos \theta_1 \tag{7}$$

or

$$2\theta_1 - 2 \sin \theta_1 \leq 1 \cos \theta_1 \tag{8}$$

This equation (8) can be solved for θ_1 and has the solution

$$\theta_1 \leq 80.3° \tag{9}$$

The total angle AOC must satisfy the following condition:

$$\measuredangle AOC \leq 160.6° \tag{10}$$

If tangents are drawn to the arc $\overset{\frown}{ABC}$ at points A and C (Fig. 2), they will intersect at point D. In terms of the angle of intersection $\measuredangle ADC$,

$$\measuredangle ADC \geq 19.3° \tag{11}$$

Since

$$\measuredangle ADC + \measuredangle AOC = 180°.$$

DISCUSSION

Equation (11) is an alternate form for expressing the "rule" given in Equation (1), and one which can be applied in practice by simple measurements. To accomplish this, reference is made to Fig. 2, where it is seen that a simple measurement of the angle formed by the intersecting tangents suffices. In practice, an upper model of a case in question is indexed at the level of the contact points of the upper right and left mesiopalatal areas of the cuspid teeth. A third point, determined by the contact-point level of the two most forward-positioned teeth on the arc, is then chosen. These three points are transposed and the segment of the circle which they determine is drawn. Two intersecting tangents are then projected from these points and the angle between them measured by an ordinary protractor.

Those arches in which the malpositioned dental units comprise the upper anterior segment, having angular measurements at the point of intersection of the tangents not greater (equal to or less than) than 19.3 degrees, must represent extremely narrow arch form. Arches of this type with the associated findings of failure in lateral development on the posterior segments, additionally presenting a high narrow vault, are recognized as having in their

etiologic history the factors underlying abberation in growth and development. This over-all problem must be approached orthodontically whether or not complicated by superimposed periodontal disease and one of its natural sequela, the pathologic migration of teeth.

CONCLUSION AND SUMMARY

In essence, experience has suggested a clinical "rule" which logically takes the form of an assumption or explanatory hypothesis in any theory. This hypothesis is formalized under given conditions, made explicit, and developed with the aid of the principles of geometry to render an alternate measuring procedure which can easily be applied by the dental practitioner.

A NEW MOUTH PROP FOR ORAL EXAMINATIONS AND OPERATIVE PROCEDURES IN RODENTS

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IN ORDER to facilitate oral examinations and operative procedures in live rats and other rodents, a new intraoral mouth prop has been developed. This device has several advantages over apparatus designed previously for such purposes.[1, 2, 3] Since the mouth prop displaces tissues in the horizontal as well as the vertical plane, it not only separates the jaws, but also spreads the cheeks apart, leaving all the oral structures directly accessible (Fig. 1). Moreover, it contains no encumbering extraoral components which might obstruct light and interfere with operative manipulations. Therefore, use of the device simplifies problems related to visibility, photography, and intraoral procedures, and leaves the whole animal free for gross manipulation. Another advantage is that the appliance does not open the mouth maximally, and hence offers little or no obstruction to respiration.

The mouth prop consists of upper and lower wire frames joined by right and left elbows (Fig. 2). The upper and lower frames contain loops to engage the incisors and keep the jaws apart, and the elbows contain extensions to push the cheeks outward. Insertion is accomplished with the anesthetized rat lying on its back and its upper incisors looped by a small piece of string tied to a stationary hook. The jaws are opened by pulling the lower incisors away from the stationary hook, and the prop is inserted in two stages. First, the elbows are compressed and introduced into the right and left vestibules of the mouth and at the same time the lower incisors are hooked into the appropriate wire loop. Then, the elbows are pushed to the posterior portion of the vestibules by a rotary movement and the upper incisors are hooked into the upper incisor loop. The elbows of a well-fitted appliance extend to the posterior limits of the vestibule and expose the buccal surfaces of all the molars. Once the device is in place, the loop of string can be removed and the animal manipulated freely.

In constructing the mouth prop, materials and methods routinely used in the average orthodontic laboratory are employed. Round, stainless steel orthodontic wire, 0.025 inch in diameter, seems to offer the best combination of strength and resiliency for appliances to be used in young adult rats. Remaining tools and materials consist of bird-beak pliers, wire cutters, a hemostat, and soldering equipment. The approximate size of the appliance is established by taking two measurements: the distances (1) from the posterior limit of the

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vestibule to the upper incisors, and (2) from the posterior limit of the vestibule to the lower incisors. In the rat, this length in the upper jaw is greater than that in the lower. These measurements are used for reference in determining the over-all length of the arms and in placing the incisor loops. A suitable mouth opener with only one upper and one lower incisor loop can be made

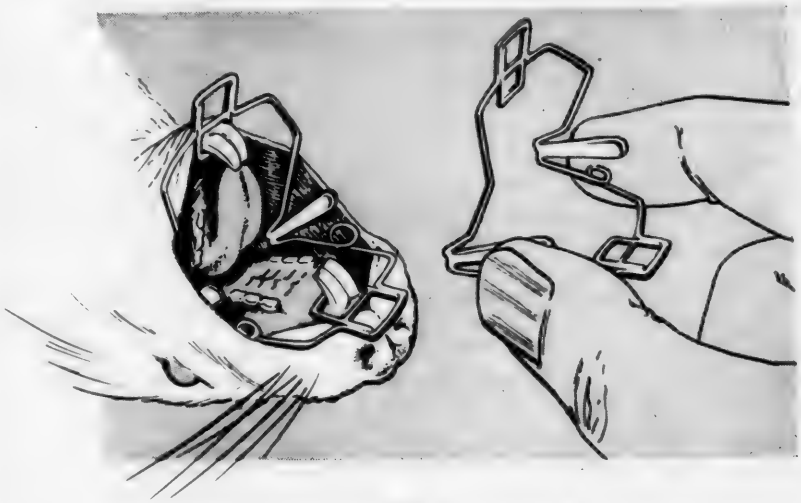

Fig. 1.

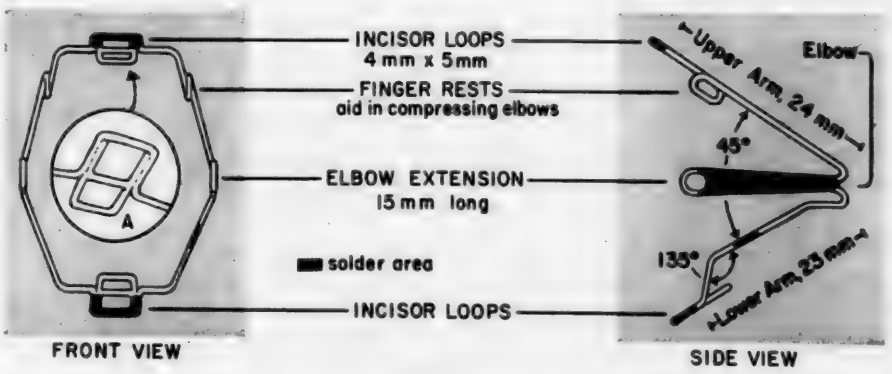

Fig. 2.

from a single length of wire. Since head size varies much less than body size, such an appliance can be used for most purposes in rats varying widely in body size. However, by using an appliance with two incisor loops at each end (see Fig. 2, front view, inset *A* for details), a more accurate fit can be achieved in rats of different weights. For example, the device depicted in Fig. 2 affords excellent exposure of the complete molar dentition of rats weighing from 200 grams to 450 grams.

With slight modification, devices of the same basic design can be used successfully in other rodents besides the rat. An opener of similar proportions, but of thinner wire, is suitable for a mouse appliance, whereas one for hamsters requires opening the elbow angle to about 70 degrees and using arms of equal length. With a device made for rats, it is possible to obtain access sufficient for certain purposes to the molar regions of guinea pigs.

The new rodent mouth prop has been successfully used to examine more than 100 rats weighing from 70 to 600 grams, a number of hamsters and guinea pigs, and a few mice. It has also been useful in studies in which it has been necessary to apply drugs to specific oral sites, to obtain bacteriologic specimens, and to perform surgical procedures, such as the removal of teeth, the excision of frena, and the extirpation of the minor sublingual glands. Confining the rodent for a few minutes in a jar containing trichloroethylene has provided anesthesia adequate for short procedures, whereas Nembutal and atropine have been used for longer operations.

The author is grateful to George Marsden for preparing the illustrations.

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THE CARCINOGEN 20-METHYLCHOLANTHRENE AND SALIVARY GLAND TRANSPLANTS

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INTRODUCTION

LITTLE information is available concerning salivary gland transplants and known carcinogens.

Species-specific viruses and their effects on transplants of salivary glands were dealt with in a recent communication[1]; and effects of these virus strains were also reported with regard to tooth germ transplants.[2] Behavior of tooth germ transplants with this virus was similar, in many ways, to findings with tooth germs transplanted with polycyclic hydrocarbon carcinogens.[3, 4, 5] The effect of carcinogens on salivary gland transplants would therefore be of interest, particularly to determine if there is any similarity to results obtained with the virus and other tissue transplants. Furthermore, since the origin of salivary gland disease is often obscure, such a study could offer particular points of information. This communication represents a report on the reaction of near-term guinea pig salivary gland transplants to 20-methylcholanthrene.

METHODS

Salivary gland tissues were obtained from near-term guinea pig fetuses and homotransplanted to the eyes and brains of young adults. Seventy-five hosts received untreated transplants (32 to the eyes and 43 to the brains). These explants of salivary gland tissues served as controls. A total of 184 hosts received 20-methylcholanthrene treated tissues (47 to the eyes and 137 to the brains).

Following removal from donor fetuses, salivary gland tissue was immersed at once in normal saline and afterward cut into pieces small enough to be easily drawn up into a 17 or 18 gauge trocar. Failure to keep chilled, and to prepare this salivary gland tissue at once, resulted in sticky masses that were hard to handle. Unless this was done, the pieces adhered to almost any surface and were not easily expelled from the trocars. The method of treating tissues with the carcinogenic hydrocarbons has been described previously.[3, 4, 5] In brief, however, no more than several crystals of 20-methylcholanthrene were placed on each piece of tissue just before transplantation.

During the course of these experiments, animals were checked three times weekly. Tissue transplants were allowed to remain in place from 23 to 176

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days before recovery. Recovered explants were fixed in formalin, sectioned, and stained with hematoxylin and eosin for histologic study.

RESULTS

In the present experiments, the methylcholanthrene-treated eye transplants of salivary gland tissue were not successful but the brain transplants were. Occasionally, treated transplants would survive for about 30 days in the anterior chamber before complete resorption was accomplished and this was usually preceded by marked inflammatory reactions. Untreated eye transplants survived at this site but underwent a more gradual resorption without any accompanying inflammatory response. By 60 days, after an earlier period of growth, not much of these transplants remained.

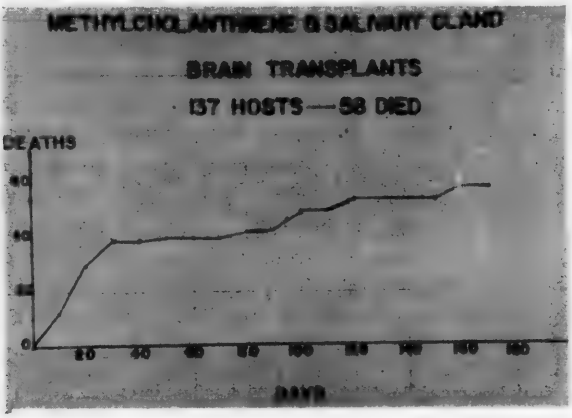


Chart A.

Death of animals with brain transplants of methylcholanthrene-treated salivary gland tissue was greater than expected in the first 30 days following transplantation. During the first 9 days, 13 hosts bearing brain transplants of carcinogen-treated salivary gland tissue died. Twenty-six additional animals died between 9 and 30 days. Following this, there was an intermission and except for three deaths, no more hosts died until the ninetieth day, and by 120 days a total of 13 had died. Then no more expired until the one hundred and fiftieth day when 5 hosts died between this time and 160 days. The remaining animals were sacrificed. Deaths of animals with brain transplants are depicted in Chart A. Guinea pigs bearing nontreated transplants, with the exception of occasional deaths, did not have a significant mortality rate.

An example of the tissue that was transplanted in these experiments is shown in Fig. 1. This was obtained from a guinea pig fetus at about 68 days of gestation. Lymphoid tissue was often included within the tissue transplanted and a small area of such tissue is depicted at the right center margin of Fig. 1. This could not be helped because of the close association of lymph

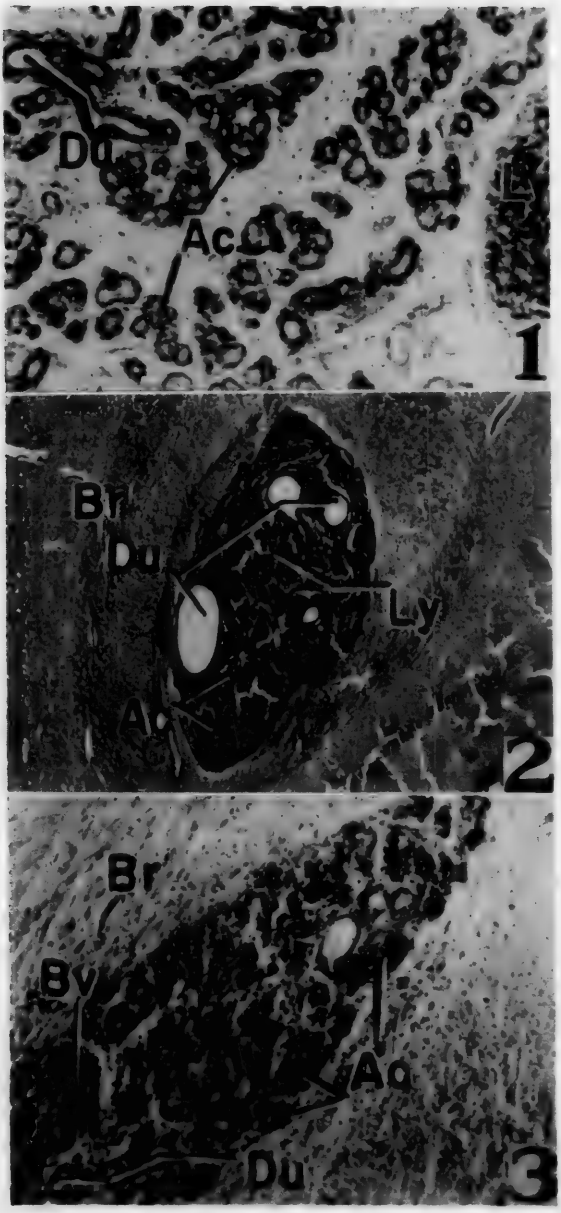
glands with this tissue. In this figure, glandular acini are set in a loose stroma of young connective tissue. Main and branching ducts are also seen. This glandular tissue does not appear to have reached maturity and thus could not have been active at transplantation time.

Brain transplants of this near-term salivary gland tissue survived and exhibited growth and development. After 35 to 40 or more days, the ducts often become distended and this is shown in the control transplant depicted in Fig. 2. This transplant was recovered after 59 days in the brain of a male guinea pig. The central portion of this explant shows considerable lymphoid tissue. Toward the bottom of this figure, groups of acinar cells can be seen. It should be noted that this explant is confined within a thin connective tissue capsule as is the transplant shown in the next figure. Fig. 3 represents another brain transplant of untreated salivary gland tissue. This was recovered after 90 days in the brain of a female guinea pig. In this instance no lymphoid tissue was observed; however, patent blood vessels are seen. There is also no large distention of ducts.

Fig. 4 shows a recovered brain transplant that was treated with methylcholanthrene just prior to explantation. This tissue was allowed to remain in the brain of a male guinea pig for 59 days before recovery. In this figure there is shown, in the upper center, a large area of acinar cell groups. There is also lymphoid tissue present. Below and to the right is an area of keratin. To the left of the keratin there is a large distended duct with a thick border of stratified squamous epithelium. In Fig. 5, a higher magnification of Fig. 4, the acinar groups of salivary gland cells can be seen to better advantage. Here, it should be noted, there is no sharp demarcation between parts of this explant and adjacent brain tissue.

Fig. 6 shows a recovered brain transplant of methylcholanthrene-treated salivary gland tissue after 90 days in the brain of a female guinea pig. Along the bottom of the transplant that is shown in Fig. 6, it is apparent that adjacent brain tissue is being invaded. In Fig. 7, an area of the transplant is shown at a higher magnification. Arrangements of acinar groups of cells and other associated cells can be seen. Fig. 8 shows an area of this transplant at a still higher magnification. There are a number of cells in various stages of mitosis. The nuclei of other cells take a deep hematoxylin stain and they vary greatly in size and shape. This anaplasia is suggestive of malignant change.

In Fig. 9 a recovered methylcholanthrene-treated transplant is shown after 162 days in the brain of a male guinea pig. There is little evidence of actual glandular tissue or acinar cell groups remaining, but keratin is abundant. As shown in Fig. 10, this keratin, as well as associated cells, presents a bizarre appearance with a direct invasion of adjacent brain tissue. In Fig. 11, which is a higher magnification of Fig. 10, cells with hyperchromatic nuclei are found in a purposeless arrangement. Several are in mitosis and there is considerable variation in their size, shape, and staining. This morphologic picture was usually found in areas of transplants after approximately 90



Key to Figures

Ac = Acinar cell groups; *Br* = brain; *BV* = blood vessels; *De* = debris; *Du* = ducts; *K* = keratin; *Ly* = lymphoid centers; *Mi* = mitotic figures; and *Tsp* = transplant.

Fig. 1.—Salivary gland tissue from near-term guinea pig embryo. (Orig. mag. ×155.)

Fig. 2.—Untreated near-term guinea pig salivary gland tissue transplant recovered after 59 days in the brain of male guinea pig. (Orig. mag. ×42.5.)

Fig. 3.—Untreated near-term guinea pig salivary gland tissue transplant recovered after 90 days in the brain of female guinea pig. (Orig. mag. ×245.)

days. Some cells shown in Fig. 10 and 11 are participating in keratin formation but many others are not. These latter cells present an extremely bizarre appearance and also suggest a malignant change.

DISCUSSION

From these studies it is evident, in the present instance, that most of the cellular events taking place are in keeping with previous investigations with chemical carcinogens and tooth germ transplants.[3-6] In the transition from the

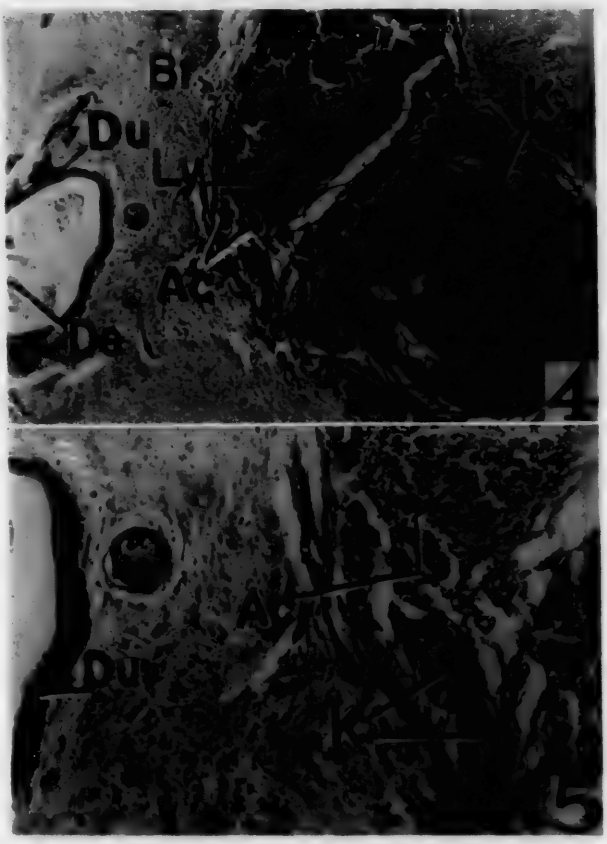


Fig. 4.—Methylcholanthrene-treated near-term guinea pig salivary gland tissue after 59 days in the brain of a male guinea pig. (Orig. mag. ×85.75.)
Fig. 5.—Higher magnification of central area of Fig. 4. (Orig. mag. ×245.)

normal to the abnormal or so-called malignant, there are, however, certain differences that were not observed in previous studies with carcinogens nor with salivary gland or tooth germ transplants made with the Shope papilloma virus.[1-6]

In these studies now being reported, host responses to anterior chamber transplants of methylcholanthrene-treated salivary gland tissue were not favorable. The marked inflammatory reactions observed did not occur with

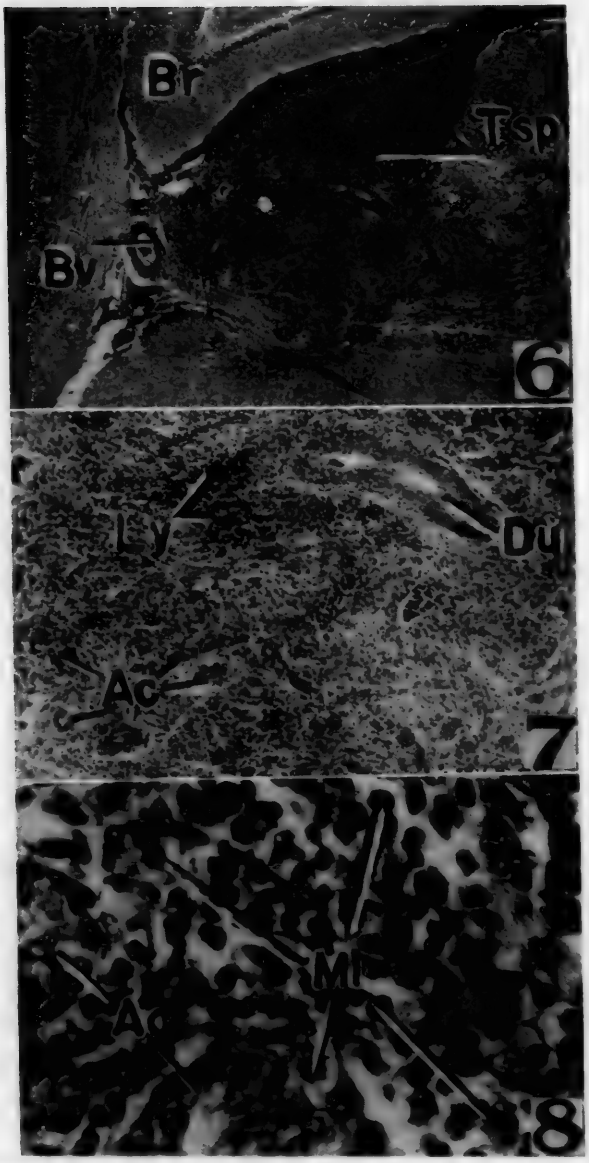


Fig. 6.—Methylcholanthrene-treated late-term guinea pig salivary gland tissue after 90 days in the brain of a female guinea pig. (Orig. mag. ×85.7.)
Fig. 7.—Higher magnification of Fig. 6. (Orig. mag. ×245.)
Fig. 8.—Higher magnification of an area in Fig. 7. (Orig. mag. ×1.102.)

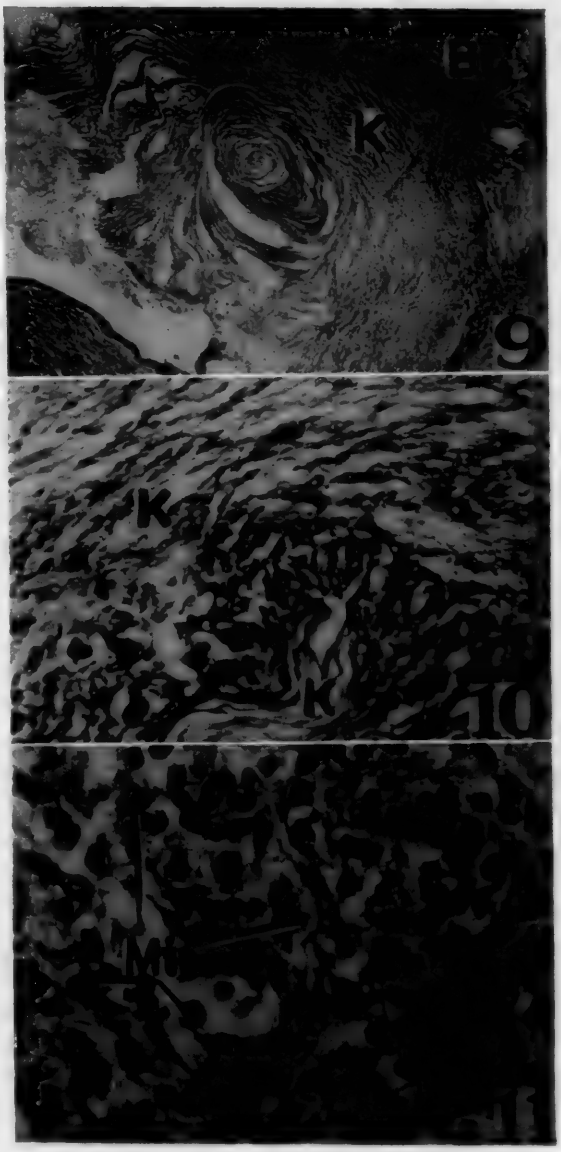


Fig. 9.—Methylcholanthrene-treated late-term guinea pig salivary gland after 162 days in the brain of a male guinea pig. (Orig. mag. ×43.)

Fig. 10.—Higher magnification of Fig. 9. (Orig. mag. ×245.)

Fig. 11.—Higher magnification of Fig. 10. (Orig. mag. ×1,102.)

similarly treated eye transplants of tooth germs.[3, 4, 5] Vigorous inflammatory responses, however, were more evident where castrates received methylcholanthrene-treated tooth germs.[6] Perhaps the carcinogen-protein complex of this salivary gland tissue had the specific ability to provoke a more vigorous antigenic host-response in the anterior chamber than was ordinarily attained with similarly treated tissue transplants of other types. It is implied that, following an initial period of latency, tissue response to methylcholanthrene is one of growth stimulation and/or overrepair of damaged parts.[7] According to Green,[8, 9] this cell proliferation is likewise a concomitant reaction of immune responses to carcinogens. It has likewise been proposed that, following the carcinogen-protein interaction, there takes place a rearrangement of electrons which in the electron-transfer processes is presumed to initiate carcinogenesis.[10] Thus, the initiating mechanisms of the carcinogenic hydrocarbons, while causing an accelerated growth, could additionally have been responsible for an alteration in the secretory by-products of the glandular cells. A similar reaction could have transpired with brain transplants which may have been responsible for the high number of deaths taking place between 10 and 30 days. In previous experiments with tooth germ transplants and dibenzanthracene, there was an initial mortality not similarly observed with methylcholanthrene and tooth germ brain transplants.[3, 4, 5] It is known that the active centers of the aromatic hydrocarbons of carcinogenic proclivities have different positions which relate to their characteristics.[11] It is also possible that the same carcinogen may likewise induce different responses with different tissues. Furthermore, the same tissues might well provoke different reactions at a particular transplantation site with different carcinogens.

It is difficult to determine exactly whether the lymphatic tissue present accompanied the donor tissue or was a lymphocytic reaction from the host. Since this tissue was usually in close association with the gland tissue, it could not be easily eliminated at the developmental stage attained when the transplantations were made. The fact that it was frequently present during the earlier periods following transplantation and was not during the later period is important. Since this lymphatic tissue was present during the earlier periods following transplantation, it presence could have been due to a host antigenic reaction. However, if its presence did not originate from a host response, then it presumably accompanied the transplanted salivary gland tissue. In consideration of this last possibility, the effect of its presence under these latter conditions could have altered any immune reaction of the host by increasing or decreasing it.

Treated transplants showed three different types of reactions in guinea pig brains. Distention of ducts and increases in the layers of ductal epithelium were usually observed. Duct distention could have resulted from accumulations of glandular secretions and also from the cellular debris, which was generally observed within the distended ducts. The second most prominent reaction was survival and proliferation of acinar groups. In places, these cells underwent anaplasia and lost all morphologic resemblance to their progenitors. In such instances, these cells lay within areas of the transplants where no sharp

demarcation was observable between the transplant and adjacent brain tissue. Hyperchromaticity of these cells was marked. In addition, the numerous mitotic figures and bizarre cellular shapes suggested anaplasia and/or malignant change. It is significant that such changes were more frequent in these experiments than had been encountered in former experiments with the carcinogens.[3-6] The third most prominent finding was the keratin formations. As observed in previous experiments, this change was customary where carcinogens had been employed. Unlike the findings with the Shope papilloma virus and salivary gland transplants,[1] keratin formations were abundant in these experiments and thus, in this respect, coincided to a greater degree with those findings with carcinogens and tooth germ transplants in noncastrates.[3, 4, 5] During the first 60 days, keratin was not prominent but increased in amount so that by 90 days and upward it figured prominently in recovered transplants. It was also noted that the keratin became markedly proliferative from 90 to 100 days. This may have been associated with causes of the renewed mortality among the host-animals at these times.

SUMMARY

Salivary gland homotransplants, treated with 20-methylcholanthrene, failed to survive in the eyes of guinea pigs. These transplants survived better in the brains of guinea pigs, after an initial period between 10 to 30 days, when a number of animals with carcinogen-treated brain transplants became moribund and died. In the brains of guinea pigs, these methylcholanthrene-treated transplants showed ductal distention and proliferation of ductal epithelium. Acinar groups showed an increase in number and, after a period of 60 days, there was anaplasia of these cells. There were also areas of keratinous proliferation and invasion of adjacent brain tissue. There was malignant transformation which, in the present experiments, took place more frequently than had been experienced with carcinogens and tooth germ transplants.

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RESEARCH ANNOTATIONS*

A NOTE ON THE OCCURRENCE OF ACTINOMYCES BOVIS IN THE HAMSTER

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IN the course of experimental production of mucoceles in the hamster, the authors discovered a tumor-like growth in the submaxillary gland of one of the animals in which the duct was tied for 3 days. Gross examination revealed a 1.5 cm. enlargement that in cross section appeared to have several foci of softening. This was clinically interpreted to be "foci of necrosis in a gland." Microscopically, these areas were pockets filled with purulent material in which a number of the ray fungus could be easily demonstrated. Because of the similarity of *Actinomyces bovis* with *Actinobacillus lignieresi* (Sautter, J. H., Rowsell, H. C., and Hohn, R. B.: North Am. Vet. 34: 341, 1953), a gram-negative organism, a tissue gram stain (Brown, J. H., and Brenn, L.: Bull. Johns Hopkins Hosp. 48: 69, 1931) was employed to differentiate between these causative agents. The colonies proved to be gram-positive. The origin of the organism in this case was unknown. (See Figs. 1 and 2.)

Fig. 1.

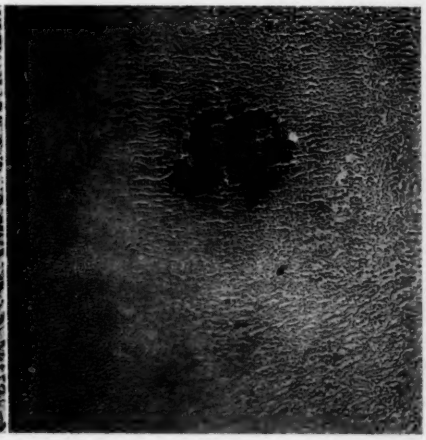

Fig. 2.

Fig. 1.—Photomicrograph of *Actinomyces bovis* colony surrounded by polymorphonuclear leukocytes. Note also formation of granulation tissue surrounding the pocket of pus. (Hematoxylin and eosin stain; orig. mag. ×150.)

Fig. 2.—Photomicrograph of tissue gram stain (Brown-Brenn) demonstrating gram-positive staining of colony. (Orig. mag. ×150.)

Knowing that repeated investigations (Wright, J. H.: J. Med. Res. 13: 349, 1905; Emmons, C. W.: Public Health Med. & Hyg. 40: 226-232, 240, 246, 1937; Rosebury, T., Epps, L. J., and Clar, A. R.: J. Infect. Dis. 74: 131, 1944) into the use of small laboratory animals for actinomycosis experimentation have met with generally uniform failure, a diligent search of the literature, both medical as well as veterinarian, was made to determine the spontaneous and/or experimental occurrence of this infection in the hamster. Although failing to find evidence of its spontaneous occurrence in the hamster, it was noted that success had been attained in the experimental production of the disease in this animal. Hazen and her group (Hazen, E. L., Martin, A. J., and Dogan, E.: New York State Dept. Health, Ann. Report 78: 1947; Hazen, E. L., Little, G. N., and Resnick, H.: J. Lab. & Clin. Med. 40: 914, 1952) have been able to produce the typical clinical infection in 75 per cent of their young (3-4 weeks) hamsters. Because of the oral aspects of actinomycosis, this spontaneous case and other experimental work are brought to the attention of dental investigators.

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WHOLE SALIVA VOLUME, SODIUM, POTASSIUM, AND CHLORIDE: DAY-TO-DAY COMPARISONS UNDER IDENTICAL AND DIVERSE FORMS OF STIMULATION

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WHOLE saliva samples were collected from 270 healthy males between 17 and 22 years of age, each individual being sampled at least twice. Chloride was determined by a modification of the method of Sendroy (Van Slyke and Hiller, J. Biol. Chem. 167: 107, 1947) and sodium and potassium were measured on a Beckman DU flame photometer (Shannon, J. D. Res. 37: 401, 1958). In the day-to-day reproducibility studies, samples were collected at 9:00 A.M. on 2 successive days from 177 subjects. Of these participants, 112 were sampled each day without exogenous stimulation, while 65 were stimulated with three large (size 32) pure rubber bands.

With no exogenous stimulation, significant ($P < 0.01$) differences were found in the means for volume, potassium, and chloride for the 2 successive days. Volume increased from 5.8 to 6.8 ml. per 5 min., potassium decreased from 22.4 to 21.9 mEq. per liter, and chloride decreased from 23.5 to 22.9 mEq. per liter. The sodium means of 9.7 and 9.5 mEq. per liter were not significantly different. Under three rubber-band stimulation, no significant differences were found for any of the variables tested. Means for volume were 9.1 and 9.4 ml. per 5 min.; for sodium, 22.3 and 22.5 mEq. per liter; for potassium, 25.5 and 25.4 mEq. per liter; and for chloride, 29.6 and 29.9 mEq. per liter.

An additional 93 subjects were sampled at 9:00 A.M. on each of 3 successive days, a different form of stimulation being employed in the collection of each sample. No exogenous stimulation, three rubber bands, and five rubber bands, respectively, were used on Days 1, 2, and 3. With the increasing strength of stimulation, significant differences ($P < 0.01$) were found for the differences between the mean values for the 3 days for volume, sodium, and chloride. Volume increased from 4.9 to 10.6 to 14.2 ml. per 5 min.; sodium increased from 8.2 to 23.6 to 26.9 mEq. per liter; and chloride increased from 25.1 to 31.8 to 32.7 mEq. per liter. The differences between the mean values for potassium, 21.0, 20.8, and 21.2 mEq. per liter, were not significant.

It was thus demonstrated that day-to-day reproducibility for the variables studied varied with the stimulation employed, and that the three rubber-band method could be recommended as a satisfactory means of reducing variability when subjects were sampled on successive days. A positive correlation was found between volume and chloride and between volume and sodium while potassium was found to be independent of flow rate. These flow rate correlations are quite clear cut when there is a large flow rate differential, as in the present study, but when the ranges of secretion rates are small, these correlations become quite obscure. At lower rates of flow we found a definite negative correlation between volume and potassium (Shannon, J. D. Res. 37: 391, 1958) both for males and for females. For chloride, we observed that, when two forms of stimulation were compared, the more intense elicitation usually led to higher chloride values but that, when correlation coefficients were calculated for the same subjects under only one form of stimulation, the coefficients were quite unpredictable (Shannon and Prigmore, Proc. Soc. Exper. Biol. & Med. 97: 825, 1958). Sodium and volume are generally conceded to be positively correlated but, again, it was our experience that this correlation is not always significant, and that stimulus intensity modifies response predictability (Report 58-49, School of Aviation Medicine, USAF, February, 1958). In all salivary studies concerned with sodium, potassium, and/or chloride, it thus becomes the responsibility of the investigator to establish quite clearly that flow rate influences have received proper consideration in data interpretation.

The opinions expressed herein are those of the authors and are not to be construed as representing official USAF policy or opinion.

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THE IMMUNOHISTOCHEMICAL LOCALIZATION OF PLASMA PROTEINS IN HUMAN GINGIVAE

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FLUORESCENT-LABELLED antibodies have found increasing use as highly specific and sensitive histochemical reagents for the detection and discrete localization of infectious agents, injected foreign antigens, and antigenic substances native to the tissues (Coons, A. H.: Internat. Rev. Cytol. 5: 1, 1956). With this immunohistochemical technic, plasma albumin, gamma globulin, and fibrin(ogen) have been demonstrated in blood vessels, lymphatics, and the connective tissue ground substance and interstitial spaces of normal, human tissues (Gitlin, D., Landing, B. H., and Whipple, A.: J. Exper. Med. 97: 163, 1953). In a number of diverse inflammatory pathoses, these plasma proteins exhibit typical patterns of increased tissue localization. Thus, plasma albumin is predominate in non-specifically inflamed tissues; gamma globulin predominates in the lesions of serum sickness and of active rheumatic carditis, rheumatoid arthritis, systemic lupus erythematosus, glomerulonephritis, periarteritis nodosa, and secondary amyloidosis; and fibrin deposits are characteristic of Shwartzman's phenomenon and thrombotic thrombocytopenic purpura (Mellors, R. C., and Ortega, L. G.: Am. J. Path. 32: 455, 1956; Vazquez, J. J., and Dixon, F. J.: Lab. Invest. 6: 205, 1957; and A. M. A. Arch. Path. 66: 504, 1958). In view of the differential localization of albumin, globulin, and fibrin(ogen) in various pathoses and the pathogenetic implications, it seemed desirable to determine the pattern of protein localization in normal and inflamed human gingivae.

Technics employed in this study included those of Coons and Kaplan (J. Exper. Med. 91: 1, 1950) together with certain modifications and the controls used by Vazquez and Dixon (J. Exper. Med. 104: 727, 1956). Gamma globulin fractions of rabbit antisera to human albumin, gamma globulin, and fibrinogen (Cutter Laboratories, Berkeley, Calif.) were labelled with fluorescein isothiocyanate according to the method of Marshall, Eveland, and Smith (Proc. Soc. Exper. Biol. & Med. 98: 898, 1958). Gingival biopsies from 10 clinically normal subjects and from 10 patients presenting for dental extractions, with moderate to advanced inflammatory periodontal disease, were secured by the technic of Ziskin, Loughlin and Siegel (Am. J. Orthodont. & Oral Surg. 30: 758, 1944). The frozen tissue specimens were stabilized for cutting on previously moistened cellophane dialysis film in a modification of the method of Bush and Hewitt (Am. J. Path. 28: 863, 1952). Cetyl alcohol (0.25 per cent w/v), added to either the ethanol-ether or 95 per cent ethanol fixatives, facilitated adhesion of the sections to the slides. The appropriately stained and mounted sections were examined microscopically for specific fluorescence. Visual comparisons were made of the intensity of staining.

Both normal and inflamed gingival tissues possessed the geographic distribution of plasma proteins reported by Gitlin and associates for the tongue and skin of normal subjects. In both normal and inflamed tissues, albumin was the consistently predominant plasma protein, with gamma globulin and fibrin(ogen) also present in significant but distinctly lesser amounts. Fluorescence associated with fibrin(ogen) often approached, and occasionally exceeded, that observed with gamma globulin. Inflamed tissue exhibited no conspicuous relative increase in either globulin or fibrin(ogen) and therefore the histochemical evidence in advanced inflammatory periodontal disease is not reminiscent of the patterns found in either serum sickness or Shwartzman's phenomenon or their presumed pathogenetic congeners. Further studies should be made of the localization of plasma proteins in the periodontal membrane and alveolar bone.

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